201-15220

Chevron Phillips Chemical Company LP

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April 29, 2004

US EPA
Document Control Officer (DCO)
EPA East Building – Room 6428
1201 Constitution Avenue, N.W.
Washington, DC 20460
Tel: 202-564-8930

Attention: HPV Challenge Program
HPV Consortium

RE: Asphalt, Sulfonated, Sodium Salt (CASN 68201-32-1)

Dear DCO:

Chevron Phillips Chemical Company LP is pleased to submit the Asphalt, Sulfonated, Sodium Salt (CASN 68201-32-1) Test Plan.

Both "Confidential" and "sanitized" copies of the test plan and relevant IUCLID dossiers/robust summary are being provided under CBI. A letter is also being sent to the Administrator in Merrifield, VA to advise him of this submission under confidentiality to your office.

We did not chose to submit an electronic version so that confidential business information (CBI) would not, inadvertently, be published on the HPV web site. We would be glad to provide electronic version to you upon request.

Vicente Santa Cruz, Ph.D., is our technical contact and can be reached at 832-813-4787 or by email at santav@cpchem.com .

Sincerely,

Fred Marashi, Ph.D.

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201-15220

High Production Volume (HPV) Challenge Program

Asphalt, sulfonated, sodium salt
CAS Number 68201-32-1
Test Plan

Chevron Phillips Chemical Company LP
10001 Six Pines Drive
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April 2004

TABLE OF CONTENTS

LIST OF TABLES AND FIGURES

LIST OF APPENDICES

ABBREVIATIONS

ACC = American Chemistry Council
API = American Petroleum Institute
BCF = predicted bioconcentration factor
bw = body weight
CPChem = Chevron Phillips Chemical Company LP
EC = Commission of the European Communities
HPV = High Production Volume
IUCLID = International Uniform Chemical Information Dataset
Koc = organic carbon partition coefficient
Kow = n-octanol/water partition coefficient
LC_{50} = lethal concentration (to 50% of animals dosed)
LD_{50} = lethal dose (to 50% of animals dosed)
LOAELs = lowest observed adverse effect levels
NOAELs = no observed adverse effect levels
OECD = Organisation for Economic Cooperation and Development
PAH = polycyclic aromatic hydrocarbons
PDII = primary dermal irritation index
Pow = n-octanol/water partition coefficient
ppm = parts per million
R = asphalt-based complex alkyl aromatic hydrocarbon mixture
SARA = Saturates, Aromatics, Resins, and Asphaltenes
SAS = Asphalt, Sulfonated, Sodium Salt
SIDS = Screening Information Data Set
USEPA = United States Environmental Protection Agency

I. EXECUTIVE SUMMARY

Chevron Phillips Chemical Company LP (CPChem) is committed to fulfilling the High Production Volume (HPV) commitments it made under the U.S. Environmental Protection Agency (USEPA) HPV Challenge Program. As part of this commitment, CPChem has volunteered to assess the health and environmental hazards, including selected physicochemical characteristics of Asphalt, Sulfonated, Sodium Salt (SAS) (CASN 68201-32-1), referred to hereafter as SAS. SAS is a drilling mud additive that is comprised of a diverse distribution of sulfonated alkylaryl hydrocarbon constituents that cross a wide range of molecular weights (500-3000) and are composed of numerous combinations of alkyl and aromatic functional groups with total carbon numbers >25. XXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXX.

CPChem has identified data from company proprietary files, peer-reviewed literature, and/or calculated endpoints using widely accepted computer modeling programs. In fulfillment with USEPA guidance for use of read-across data (USEPA, 1999b), CPChem proposes the use of surrogate data from similar structural analogues to provide additional support in our understanding of health and environmental hazards for SAS. These surrogate substances include Petroleum-derived salts of Sulfonic Acids, Asphalt, and Alkylaryl Sulfonates. Based on the available physical, chemical, environmental fate, and toxicological data for SAS, these substances demonstrate similar toxicological profiles or follow predictable trends, thus strengthening their use as surrogates.

Physicochemical endpoints for SAS are generally fulfilled by using existing measured data or data calculated by the EPIWIN© computer model. However, additional water solubility testing (per OECD Guideline 105) is proposed for this program. A review of the existing data for SAS and its related structural surrogates shows that sufficient data are available to characterize environmental fate and aquatic toxicity. Level III fugacity modeling predicts that releases of SAS to water would remain in water, releases to soil would remain in soil, and releases to air would partition primarily to soil. Ready biodegradation testing showed that SAS is not readily biodegradable and for additional perspective, SAS has low potential for bioaccumulation in the environment as demonstrated by low predicted octanol solubility, log Pow (n-octanol/water partition coefficient), and fish bioconcentration factors. Acute fish, daphnid, and algal endpoints for SAS are fulfilled with valid study data and demonstrate minimal to low toxicity to aquatic organisms. No additional testing is proposed for environmental fate and ecotoxicity.

Overall, available mammalian toxicity data on SAS (and its structural surrogates that represent many of the SAS functional groups and encompass the most toxicologically significant SAS constituents) indicate a low order of toxicity. SAS has only been tested for acute toxicity via the oral route, where results are of a similar order of magnitude for both SAS and all of the structural surrogates included in this test plan. Acute dermal and inhalation toxicity results for the various structural surrogates likewise demonstrate a low order of toxicity for this class of materials. No additional acute toxicity testing is proposed for this program.

No repeated dose studies on SAS were identified, however, multiple repeated dose toxicity studies are available for SAS surrogates encompassing the alkyl aryl, naphthenic, and asphalt functional groups. In general, results indicated a low order of repeated dose toxicity by the dermal and inhalation routes, however, liver effects in the oral study on Naphthenic Acid indicated that the liver may be a target organ. Neither SAS nor any of its structural surrogates have been tested for reproductive and developmental toxicity. To provide definitive data for SAS, CPChem proposes an OECD Guideline 422 "Combined Repeated Dose Toxicity Study with the Reproduction/Developmental Toxicity Screening Test".

Genotoxicity data exist for all three structural surrogates, Petroleum-derived Sulfonic Acids, Naphthenic Acids, and Asphalts. However, no specific genotoxicity data is available for SAS. CPChem proposes to conduct an AMES Test (OECD 471) to further support the use of surrogate data presented in this test plan.

Table 1 summarizes the available data for SAS and its structural surrogates.

Table 1. Matrix of Available and Adequate Data on SAS and Related Surrogates

Test	SAS	Sulphonic Acid Petroleum Salts (Sur. #1)	Naphthenic Acids (Sur #2)	Asphalt (Sur. #3)	Testing Planned ?
	Y/N				Y/N
Physical and Chemical Data					
Melting Point	Y	Y	Y	Y	N
Boiling Point	Y	Y	Y	Y	N
Vapor Pressure	Y	N	Y	Y	N
Partition Coefficient	Y	N	Y	Y	N
Water Solubility	N	N	Y	Y	Y
Environmental Fate and Pathways					
Photodegradation	Y	N	Y	Y	N
Stability in Water (Hydrolysis)	NA	NA	NA	NA	NA
Transport/Distribution	Y	N	Y	Y	N
Biodegradation	Y	Y	Y	Y	N
Ecotoxicity					
Acute/Prolonged Toxicity to Fish	Y	Y	Y	Y	N
Acute Toxicity to Aquatic Invertebrates	Y	N	N	Y	N
Acute Toxicity to Aquatic Plants (Algae)	Y	N	N	Y	N
Toxicity					
Acute Toxicity (Oral)	Y	Y	Y	Y	N
Acute Toxicity (Inhalation)	N	Y	N	Y	N
Acute Toxicity (Dermal)	N	Y	Y	Y	N

Test	SAS	Sulphonic Acid Petroleum Salts (Sur. #1)	Naphthenic Acids (Sur #2)	Asphalt (Sur. #3)	Testing Planned ?
Repeated Dose	N	Y (Inh. & Derm.)	Y (Oral)	Y (Inh. & Derm.)	Y
GeneticToxicity – *in vitro* Gene Mutation	N	Y	Y	Y	Y
Genetic Toxicity – *in vitro* Chromosomal Aberration	N	Y	Y	Y	N
Genetic Toxicity – *in vivo*	N	N	Y	Y	N
Reproductive Toxicity	N	N	N	N	Y
Developmental Toxicity	N	N	N	N	Y

NA = Not Applicable

NOTE:
The data used to characterize the OECD Screening Information Data Set (SIDS) endpoints for substances in this test plan were identified either in company proprietary files, peer-reviewed literature, and/or calculated using widely accepted computer modeling programs. Surrogates were used for read-across as defined by the USEPA (1999b). All data were evaluated for study reliability in accordance with criteria outlined by the USEPA (1999a). Only studies that met the reliability criteria of "1" (Reliable without restrictions) or "2" (Reliable with restrictions) were used to fulfill OECD SIDS endpoints. Additional data for SAS and the surrogates are also included in the IUCLID (International Uniform Chemical Information Dataset) attached in Appendix I. A more detailed discussion of the data quality and reliability assessment process used in developing this test plan is provided in Appendix II.

II. GENERAL SUBSTANCE INFORMATION

USE: SAS is solely used as an additive for drilling fluids to reduce torque and drag in drilling operations. Under normal operating and use conditions, SAS is not subjected to temperatures greater than 450° F (232°C) as may be expected with asphalts. Exposure to high temperature only occurs in aqueous solution (versus atmospheric conditions) when the SAS-containing drilling fluid is circulated down hole during drilling operations. Upon completion of drilling operations, the drilling fluid is circulated out of the hole and cools as it returns to the surface. The temperature of the drilling fluid being circulated out of the hole ranges from 100-150° F (37-66°C) when it reaches the surface. Under these conditions, fumes are not observed or expected to be emitted from SAS (CPChem Technical Communication, April 2004).

Soltex ® Additive, which uses SAS as the functional ingredient, has been approved for release to the aquatic environment based on data presented in this test plan. This product has been approved by NPDES (National Pollutant Discharge Elimination System) Discharge for oil and gas cutting discharge in Region 9 EPA Gulf Coast Guidelines (40 CFR, Part 435 (a)), and meets OSPAR Convention for the Protection of the Marine Environment in the North-East Atlantic (OSPAR ANNEX 17 (Ref. § 7.4c), Copenhagen: 26 - 30 June 2000).

CHEMISTRY: SAS is a very complex mixture produced by sulfonation of an asphalt-based alkyl aryl hydrocarbon feedstock followed by neutralization of the sulfonated hydrocarbon mixture with sodium hydroxide. This asphalt-based complex alkyl aromatic hydrocarbon mixture ranges in molecular weight from 500-3000 and contains SO_3^- functional groups.

The chemical complexity of SAS comes from the asphalt feedstock, which is naturally variable in composition, and has a wide array of chemical constituents and reactive sites for addition of sulfonic acid functional groups. Asphalt (known as Bitumen in Europe) "is the residuum produced from the non-destructive distillation of crude petroleum at atmospheric pressure and/or under reduced pressures or absence of steam" (Puzinauskas and Corbett, 1978). Asphalts are composed mainly of high-molecular-weight alkylaryl hydrocarbons with high carbon to hydrogen ratios, with carbon numbers > C25, boiling points > 400 °C, high viscosity, and negligible water solubility and vapor pressure. These asphalt alkylaryl hydrocarbons are a heterogeneous mixture of linear, branched and cyclic, saturated and unsaturated, and aromatic functional groups. Importantly, polycyclic aromatic hydrocarbons (PAH) such as benzo(a)pyrene, which are toxicologically significant, are only present in asphalt feedstock at very low concentrations (Phillips Petroleum Company, 1985). Asphalts contain much larger proportions of high-molecular-weight paraffinic and naphthenic hydrocarbons that are substituted with alkyl groups and ultimately sulfonated, which reduces their potential to exhibit PAH-like toxicity (IARC, 1985 in American Petroleum Institute [API], 2003b).

In practice, the asphalt alkylaryl feedstocks are chemically characterized by a saturates, aromatics, resins, and asphaltenes (SARA) analytical technique. Table 2 describes each of these fractions along with the approximate SARA proportions specifically used in SAS production (Witherspoon, 1962; Phillips Petroleum Company, 1985).

Table 2. Description of SARA Profile of Asphalt Feedstock

XXX	Saturates	Consist mainly of long chain saturated hydrocarbons with some branching, alkyl aromatics with long side chains, and cyclic paraffins (napthenes), with molecular weight of 500-1000.
XXX	Aromatic*	Consist mainly of substituted benzene and napthenic-aromatic nuclei with alkyl side chain constituents, with molecular weight range of 500-900.
XXX	Resins	Consist mainly of heterogeneous polar aromatic compounds with small amounts of oxygen, nitrogen, and sulfur, with molecular weight range of 800-2000. Considered lower molecular weight asphaltenes.
XXX	Asphaltenes	Consist mainly of highly condensed aromatic compounds with one or two chromophores containing 4 to 10 fused rings each, with a significant number of alkyl constituents. They have a molecular weight range of 500-1000.

*XXXXXXXXXXXXXX

When the alkylaryl hydrocarbons in the asphalt feedstock are sulfonated during production of SAS, they remain intact and the sulfonation process chemically derivitizes them, converting them into alkylaryl sulfonate sodium salts that still contain carbon

numbers >C25. The sulfonic acid functional groups form by reaction with double bonds in the hydrocarbons, whether on alkyl chains or aromatic rings. The addition of the sulfonic acid group(s) increases the molecular weight, raising melting points >350 °C and boiling points >500 °C, and further reducing vapor pressure versus asphalt. Sulfonation also increases water solubility, or for the higher molecular weight and more hydrophobic constituents, renders them readily dispersible such that they form stable colloidal dispersions or micelles in water.

Understanding that SAS is a complex chemical mixture of alkylaryl sulfonated isomers becomes critically important when characterizing SAS OECD SIDS endpoints. The physicochemical, environmental, and human health properties of SAS will be a function of the specific constituents in any given sample and should be expected to result in ranges versus discrete endpoints for some physical, chemical, and environmental fate properties. Additionally the large molecular size of most SAS constituents will reduce their bioavailability.

Note:XXXX

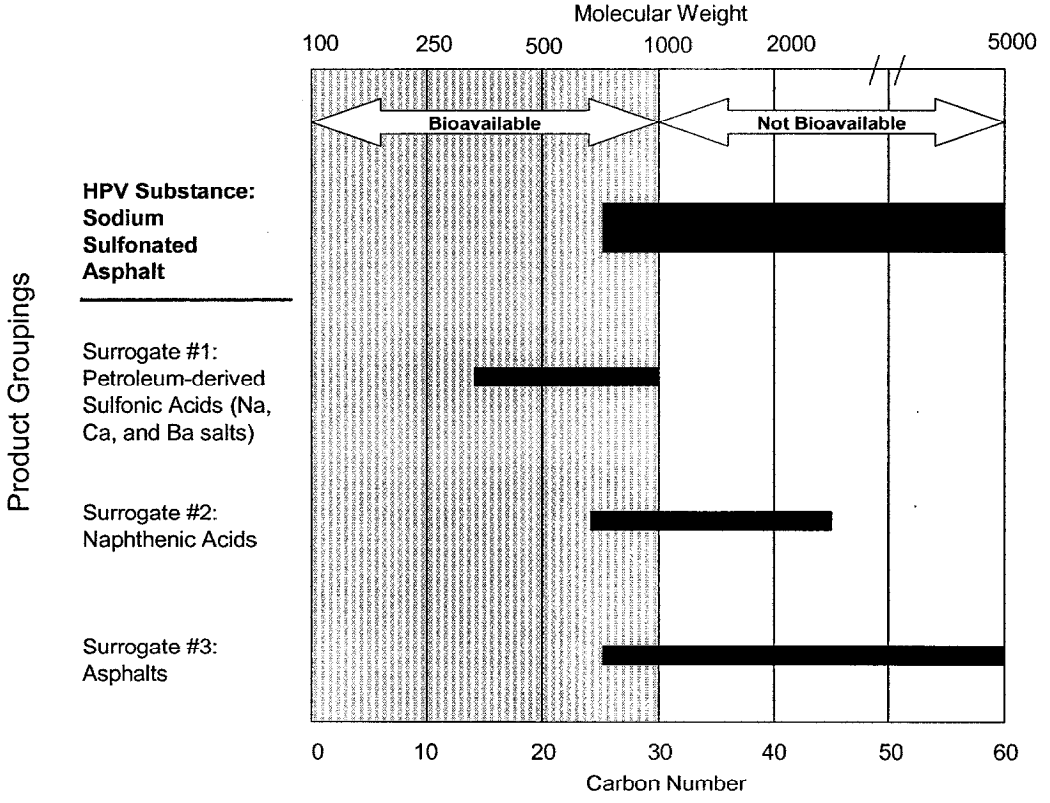
	Additives. (XXXXXX).
Structural Surrogate #1 Sulfonic Acids, petroleum salts CAS Number 68783-96-0 (sodium salt) CAS Number 61789-86-4 (calcium salt) CAS Number 61790-48-5 (barium salt)	Generic Structure: $R-SO_3^- Na^+$ or $(R-SO_3^-)_2 Ca^{+2}$ or $(R-SO_3^-)_2 Ba^{+2}$ R = alkylaryl hydrocarbon with molecular weight = 300-400 Overall Molecular Weight = 300-950 Total Carbon Number >C12-30
Structural Surrogate #2 **Napthenic Acids, Petroleum, crude** CAS Number 64754-89-8	Complex mixture, predominantly of compounds that contain carboxylic acid functional groups and five- to six-member naphthenic rings in their molecular structures. Phenolic compounds and acidic sulfur compounds may also be present. Overall Molecular Weight = 300-950 Total Carbon Number C11->C30
Structural Surrogate #3 **Asphalt Category** CAS Number 8052-42-4 CAS Number 64741-56-6 CAS Number 64742-07-0 CAS Number 64742-16-1 CAS Number 64742-85-4 CAS Number 64742-93-4	Asphalt is the residuum produced from the nondestructive distillation of crude petroleum. Asphalts are complex mixtures of hydrocarbons with molecular weights ranging from 500 to 2000. Overall Molecular Weight = 500-2000 Total Carbon Number Predominantly >C25

Understanding the complex chemical composition of SAS was critical in developing the structural surrogate strategy for this test plan. In evaluating possible structural surrogates for SAS, CPChem considered USEPA HPV criteria for choosing surrogates (USEPA, 1999b), and reviewed a wide range of petroleum-based products that contain similar functional groups to SAS. Importantly, all of the constituents in SAS are at or above the 500-1000 molecular weight range. Due to their larger molecular size and tendency to form micelles or colloids in solution, both of which inhibit biological uptake of the larger SAS molecules, the majority of constituents will not be able to cross cell membranes. In short, they will not be bioavailable to exert systemic toxicity. Regulators at both the USEPA and the European Union have acknowledged this practical molecular weight/molecular size bioavailability cut-off, and have incorporated it into technical guidance for testing and registration of chemicals (Boethling and Nabholz, 1996; EC, 2003).

Figure 1 graphically depicts the alkylaryl sulfonate distribution of SAS constituents versus carbon number and molecular weight range. It also illustrates the bioavailable

lower molecular weight range and shows that most SAS constituents have a large molecular size that will reduce bioavailability and potential for systemic toxicity.

Figure 1. Alkylaryl Sulfonate Distribution of SAS Constituents Versus Carbon Number and Molecular Weight Range (shading indicates bioavailable range)



As described above, SAS comprises a diverse distribution of sulfonated alkylaryl hydrocarbon constituents that cross a wide range of molecular weights (500-3000) and are composed of numerous combinations of alkyl and aromatic functional groups with total carbon numbers >25. The proposed structural surrogates possess these same functional alkylaryl hydrocarbon and/or alkylaryl sulfonate groups.

- **Surrogate #1 Sulfonic Acids, Petroleum Additive Alkaryl Sulfonate Category**

The Sulfonic Acids, petroleum salts category is composed of sodium, calcium, and barium salts of sulfonated alkyl aromatic hydrocarbons. In aqueous conditions the salts will be dissociated, making the same petroleum sulfonic acid ionized species from all three salts forms. This is the most significant surrogate because it overlaps the most bioavailable and therefore toxicologically significant lower molecular weight fraction of SAS constituents, and therefore represents a conservative read-across benchmark for toxicity and ecotoxicity endpoints. One SAS functional group

that is lacking in this category is the naphthenic or polycyclic aromatic sulfonated hydrocarbons, but these functional groups are overlapped by the other proposed surrogates as described below.

- **Surrogate #2 Naphthenic Acids, Reclaimed Substances: Streams Containing Naphthenic Acids Category**

The Naphthenic Acids group adds additional perspective as a read-across surrogate beyond that offered by the Sulfonic Acids, Petroleum salts. They help represent the multicyclic aromatic hydrocarbon fraction of SAS constituents, whereas Surrogate #1 only represents the alkylaryl monocyclic fraction of SAS constituents. The Naphthenic Acids are used in this test plan for read-across for physical/chemical, environmental, and mammalian endpoints. They may contain low levels of sulfonic acid functional groups, but they principally contain carboxylic acid groups, which also impart similarly increased water solubility and reactivity to the alkylaryl hydrocarbon – analogous to the impact of sulfonic acid functional groups on SAS. Overall the Naphthenic Acids represent a conservative read-across benchmark for toxicity and ecotoxicity endpoints since they have a bioavailable fraction. They are relatively nontoxic and are of less concern as a constituent that contributes toward toxicity in the overall SAS product. This illustrates the importance of focusing on the more bioavailable, lower molecular weight fraction of SAS constituents in meaningfully characterizing the SIDS endpoints for SAS.

- **Surrogate #3 Asphalt Category**

CPChem originally intended to include SAS as a member of the HPV Asphalt Category, but abandoned this effort due to dissimilarity arising from the impact of sulfonation on the physical and chemical characteristics of SAS versus nonsulfonated asphalts. However, the Asphalts Category is composed of the same alkylaryl hydrocarbons that are used to make SAS and therefore provides perspective on the environmental fate and toxicological profile of the alkylaryl hydrocarbon functional group in SAS. Importantly the Asphalts Category is predominantly nontoxic and in toxicity tests Asphalt samples were solubilized to maximize bioavailability, which again illustrates that this is a conservative structural surrogate to SAS.

SAS and surrogates #1 (Petroleum Salts of Sulfonic Acids) and #2 (Naphthenic Acids) are characterized by high melting point and boiling point ranges and very low vapor pressures. In the aquatic environment, the SAS sodium salts will be dissociated leaving the ionized species as the environmentally relevant form and surrogates #1 and #2 would also be dissociated in aqueous conditions further demonstrating their suitability as surrogates.

IV. PHYSICOCHEMICAL PROPERTIES

Importantly, SAS and its structural surrogates are complex heterogeneous mixtures containing many different sulfonated alkylaryl isomers as described above. Therefore, physicochemical properties may vary according to proportions of individual constituents in the sample tested, which results in these substances having ranges rather than discreet melting and boiling points or vapor pressures. Only limited physicochemical testing has been completed for SAS, as summarized in Table 4. Calculations using EPIWIN (USEPA and Syracuse Research Corporation, 2000) are also provided where representative alkylaryl hydrocarbon chemical structures were developed for SAS constituents in the carbon number range of C26-C41. The representative structures are presented in Tables 4a, 5a, and 5b, and are discussed further in subsequent sections.

The physical chemical data for SAS and its HPV Category surrogates provided in Table 4 were experimentally measured or calculated using EPIWIN.

Table 4. Measured and Calculated Physicochemical Properties

Physical and Chemical Data				
Test	**SAS**	**Sulfonic Acids, Petroleum Salts (Sur. #1)**	**Napthenic Acids (Sur. #2)**	**Asphalts (Sur. #3)**
Melting Point	See Table 4a	349.84 °C[1]	117 to 160 °C[3]	ND
Boiling Point	>500 °C[7]	935.88 °C[1]	233 to 375 °C[3]	>450 °C[2]
Vapor Pressure	Negligible[7]	$<1X10^{-10(1)}$	1.4×10^{-5} to $1.8 \times 10^{-3(3)}$	Negligible[2]
Kow Partition Coefficient	< 0, 1.1, 3.2, and > 6.2[5] <0[6]	ND	5.1 to 9.2[4]	≥10.0[4]
Water Solubility	ND	ND	0.0003 to 2.1[4]	ND

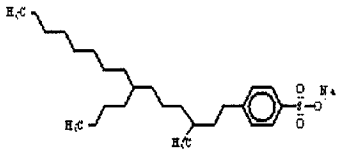
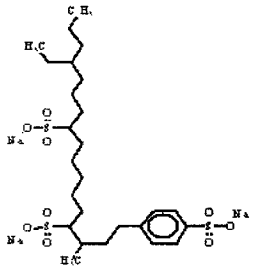
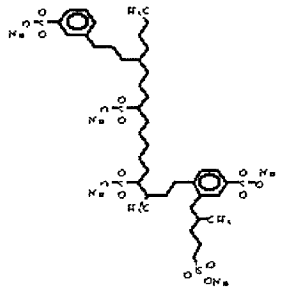
[1]ACC, 2001.
[2]API, 2003d.
[3]EPIWIN v3.10; MPBPWIN v1.40.
[4]EPIWIN v3.10; calculated using WSKOW v1.40.
[5]TNO Environmental and Energy Research (TNO), 1997.
[6]Chemex Environmental International Limited, 2003.
[7]CPChem internal communication
ND = No Data Available

To help further characterize the SAS SIDS physicochemical and environmental fate endpoints and the trends across the expected range of SAS constituents, three representative sulfonated alkylaryl hydrocarbon chemical structures were developed where carbon number and degree of sulfonation were varied. These are representative of SAS constituents across the carbon number range of C26-C40, as summarized in Table 4a and entered into EPIWIN as follows:

1. A C26 monosulfonated species representing the low end of the molecular weight range of SAS.
2. A C26 trisulfonated species representing a polysulfonated C26 constituent and to illustrate the impact of increasing sulfonation alone versus monosulfonated (#1).
3. A C40 penta-sulfonated species representing the higher end of the carbon number and sulfonic acid group substitution.

In general, increased sulfonation increases boiling points and is expected to increase melting points (EPIWIN can not estimate melting points > 349.84 °C.) Increased sulfonation will also further reduce vapor pressure. Therefore, calculated values for monosulfonated isomers are used for SAS with reporting as greater than "the monosulfonated representative structure" to indicate that there will be a range or that some fraction will decompose.

Table 4a. EPIWIN Physicochemical Data for Representative Structures

Physical and Chemical Data			
Parameter	 ($C_{26}H_{43}O_9S_3Na_3$)	 ($C_{26}H_{45}O_3SNa$)	 ($C_{40}H_{61}O_{15}S_5Na_5$)
Melting Point	> 349.84 °C [1]	>349.84 °C[1]	>349.84 °C[1]
Boiling Point	739.46 °C [1]	916.13 °C[1]	1276.77 °C[1]
Vapor Pressure	6.02×10^{-18} mmHg at 25 °C [1]	3.9×10^{-23} mmHg at 25 °C [1]	1.75×10^{-33} mmHg at 25 °C [1]
Kow Partition Coefficient	6.78 [2]	2.32 [2]	4.05 [2]
Water Solubility	0.002071 milligrams per liter (mg/L) at 25 °C [2]	0.6256 mg/L at 25 °C [2]	4.655×10^{-5} mg/L at 25 °C [2]

[1] EPIWIN v3.10; MPBPWIN v1.40.

[2] EPIWIN v3.10; calculated using WSKOW v1.40.

These EPIWIN data show that SAS and its surrogates will have high melting point ranges (>349.84 °C), boiling point ranges > 900 °C, and very low vapor pressures. SAS has a water soluble fraction and is water dispersible by design. Therefore, a water solubility continuum will occur in which SAS fractions may be soluble across a range from ppm to zero. The water solubility measurements were operationally defined and not pursuant to USEPA or Organisation for Economic Cooperation and Development (OECD) guidelines. Importantly, the functional groups in SAS constituents are not labile to hydrolysis. The high degree of sulfonation will significantly increase water solubility and reduce octanol solubility such that SAS constituents will have a very low Kow, where Kow values can range from 2 to 7, and which suggests there is low cause for concern for bioaccumulation in aquatic organisms.

Summary: The weight of evidence supports that adequate data (i.e., Klimisch rating 1 and 2) are available for most physical and chemical endpoints; additional water solubility testing is proposed for the USEPA HPV Challenge Program (see Tables 4 and 4a and IUCLID documents).

V. EVALUATION OF ENVIRONMENTAL FATE DATA

SAS and its structural surrogates are complex heterogeneous mixtures containing many different sulfonated alkylaryl isomers, as described above. Therefore, environmental fate properties will vary according to the relative proportions of specific functional groups in the sample tested, which results in these substances having ranges rather than discreet environmental fate endpoints such as rate constants, reaction profiles, or partitioning behavior. This complexity especially confounds whole SAS product fugacity modeling since SAS constituents will be subject to differential partitioning depending on the degree of sulfonation and overall carbon content, etc.

Environmental fate data for SAS were either experimentally measured or estimated using representative structures in EPIWIN, and are provided in Tables 5, 5a, and 5b.

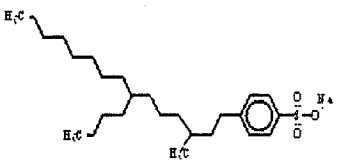
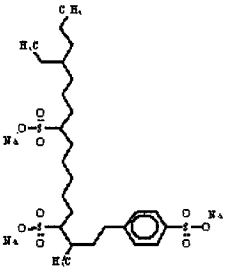
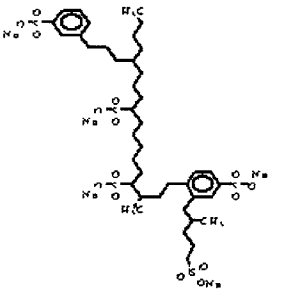
Table 5. Measured and Calculated Results for Environmental Fate and Pathways

Environmental Fate and Pathways				
Test	**SAS**	**Sulfonic Acids, Petroleum Salts Na (Sur. #1)**	**Napthenic Acids (Sur. #2)**	**Asphalts (Sur. #3)**
Photodegra-dation and Atmospheric Oxidation: • OH Half Life	See Table 5a	ND	0.3 to 0.6 days[5]	Physicochemical characteristics do not favor distribution to environmental compartments where photodegradation

Environmental Fate and Pathways				
Test	**SAS**	**Sulfonic Acids, Petroleum Salts Na (Sur. #1)**	**Napthenic Acids (Sur. #2)**	**Asphalts (Sur. #3)**
				reactions will occur.[7]
Stability in Water (Hydrolysis)	No Data, Low Potential	Low Potential[1,2]	Components do not undergo hydrolysis – no testing proposed[6]	Category does not undergo hydrolysis.[7]
Transport/ Distribution Fugacity Estimated Koc: Estimated BCF:	See Table 5b See Table 5a See Table 5a	ND	NA	Tend to remain intact and within the medium in which they were released.[7]
Biodegra-dation	3-6% in 28 days[3] 0% in 56 days[4]	8.6% biodegraded after 28 days[1]	6-7%[6]	Under realistic exposure conditions where the bulk properties of asphalt limits dispersion and the available surface area for microbial exposure, biodegradation is expected to be minimal.[7]

[1]ACC, 2001.
[2] Based on Functional Group and Chemical Class: Branched hydrocarbon chain (CAS N 61789-86-4); linear hydrocarbon chain (CAS N 68783-96-0); and sulfonic acid (CAS N 61790-48-5).
[3]TNO, 1991b.
[4]TNO, 1993.
[5]EPIWIN v3.10; calculated using AOP Program v1.90.
[6]API, 2003c.
[7]API, 2003d.
NA = Not Applicable.
ND = No Data Available.

Table 5a. EPIWIN Environmental Fate and Pathways Data for Representative Structures

Physical and Chemical Data			
Parameter	 ($C_{26}H_{43}O_9S_3Na_3$)	 ($C_{26}H_{45}O_3SNa$)	 ($C_{40}H_{61}O_{15}S_5Na_5$)
Photodegradation and Atmospheric Oxidation:			
• OH Rate Constant	28.4858 x 10^{-12} cm^3/molecule-sec[1]	31.5298 x 10^{-12} cm^3/molecule-sec[1]	45.0897 x 10^{-12} cm^3/molecule-sec[1]
• OH Half Life	4.506 Hrs[1]	4.071 Hrs[1]	2.847 Hrs[1]
Transport/ Distribution			
Fugacity	See Table 5b	See Table 5b	See Table 5b
Estimated Koc:	1.821 x 10^{6} [(2)]	9.466 x 10^{7} [(2)]	1 x 10^{10} [(2)]
Estimated BCF:	70.79[3]	70.79[3]	70.79[3]

[1]EPIWIN v3.10; calculated using AOP Program v1.90.
[2]EPIWIN v3.10; calculated using PCKOC Program v1.66.
[3]EPIWIN v3.10; calculated using BCF Program v2.14.

Summary: The weight of evidence in this test plan supports that no further testing is necessary to meet HPV SIDS endpoints relating to environmental fate. Adequate data (i.e., Klimsch rating 1 and 2) are available for all endpoints; no additional testing is proposed for the USEPA HPV Challenge Program (See Tables 5, 5a, and 5b and IUCLID documents). SAS is expected to be labile and mobile if released to the environment, but will disappear based upon both biotic and abiotic degradation mechanisms. SAS does not pose any bioaccumulation hazard, as described in detail below.

A. Photodegradation – Atmospheric Oxidation

Constituents of SAS are polyaromatic and act as chromophores (which absorb light energy in the 290 nm to 800 nm range where photolytic reactions may result). The degree and rate at which these compounds undergo direct photolysis is a function of the light intensity at site of the SAS molecules. Also, indirect photodegradation may occur in the atmosphere where the SAS constituents interact with photochemically produced hydroxyl radicals, ozone, or nitrogen oxides. Hydrocarbons, such as the alkylaryl hydrocarbons in SAS, react readily with OH^- and NO_3 radicals, and monochromatic and dichromatic compounds react readily with OH^- radicals to undergo degradative reactions (Atkinson, 1990 in API, 2003b). However, due to the fact that SAS and its sulfonated structural surrogates have very low vapor pressures, they do not have a tendency to volatilize to air where they can undergo reactions with photosensitized oxygen in the form of hydroxyl radicals (OH^-). As a result, these reactions are not expected to be significant environmental fate processes.

Values for SAS photodegradation and atmospheric oxidation were calculated based upon representative chemical structures using EPIWIN, and are shown in Table 5a. A calculated half-life for SAS of 3 to 5 hours and rate constant of 28×10^{-12} - 45×10^{-12} cubic centimeters (cm^3)/molecule-sec has been estimated using EPIWIN for reaction with hydroxyradicals.

Summary: These results show that SAS may be subject to photodegradation and atmospheric oxidation, and are sufficient for USEPA HPV Challenge Program purposes; no further testing is warranted.

B. Hydrolysis

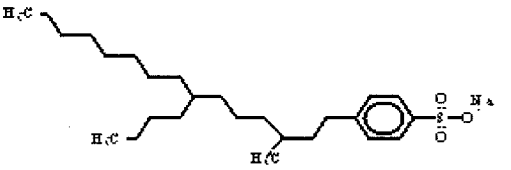
Chemicals that have a potential to hydrolyze include alkyl halides, amides, carbamates, carboxylic acid esters and lactones, epoxides, phosphate esters, and sulfonic acid esters (Harris, 1982 in API, 2003a). Because SAS does not contain significant levels of these functional groups, components in SAS are not subject to hydrolysis.

Summary: Components in SAS do not undergo hydrolysis. Existing information is sufficient for USEPA HPV Challenge Program purposes; no further hydrolysis testing is warranted.

C. Chemical Transport and Distribution in the Environment (Fugacity Modeling)

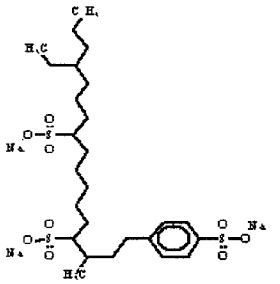
EPIWIN produced the following Level III Fugacity results for the representative structures to SAS:

Table 5b. EPIWIN Level III Fugacity Results for Representative Structures



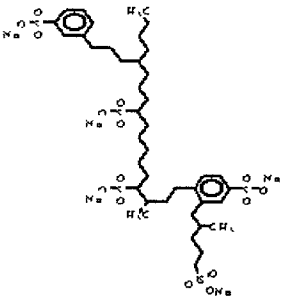
(C_{26} H_{43} O_9 S_3 Na_3)

Compartment	100% to air	100% to water	100% to soil	Equally to each compartment
Air	5.33%	0.000131%	0.00%	0.444%
Water	2.47%	12.3%	0.00161%	8.2%
Soil	74.7%	0.00183%	100%	33.1%
Sediment	17.5%	87.7%	0.0114%	58.2%



(C_{26} H_{45} O_3 S Na)

Compartment	100% to air	100% to water	100% to soil	Equally to each compartment
Air	0.0055%	0.00%	0.00%	0.35%
Water	10.3%	99.5%	5.66%	31.5%
Soil	89.6%	0.00%	94.3%	68.0%
Sediment	0.0557%	0.537%	0.0306%	0.17%



(C_{40} H_{61} O_{15} S_5 Na_5)

Compartment	100% to air	100% to water	100% to soil	Equally to each compartment
Air	0.00%	0.00%	0.00%	0.00246%
Water	3.55%	82.3%	0.156%	15.3%
Soil	95.7%	0.00%	99.8%	81.4%
Sediment	0.763%	17.7%	0.0334%	3.29%

Summary: Results from the Level III fugacity modeling indicate that releases of SAS to water would remain in water, while releases to air would partition primarily to soil. Likewise, releases to soil would remain in soil. Further fugacity modeling is not warranted for the USEPA HPV Challenge Program.

D. Biodegradation and Bioaccumulation

SAS has been tested in two Ready Biodegradation tests in seawater according to proposed EC test guidelines and was not readily biodegradable (3 to 6% in 28 days and 0% in 56 days) in both studies. The results are reliable without restrictions and fulfill the HPV SIDS endpoint for SAS.

In addition, the EPIWIN predicted bioconcentration factor (BCF) for representative structures to SAS were 70.79 and the organic carbon partition coefficients (Koc) were 1.8×10^6 to 1×10^{10}. The BCF should be low, but the BCF QSAR defaults to a low BCF for substances that are expected to be ionized in aqueous media. Overall these results indicate that SAS will be sorptive and poses a low bioaccumulation potential.

Summary: Adequate biodegradation data are available; no additional biodegradability testing is proposed for the USEPA HPV Challenge Program (See Table 5 and IUCLID documents).

VI. ECOTOXICITY DATA

Acute fish, daphnid, and algal endpoints for SAS are fulfilled with valid study data. The studies were conducted consistent with relevant OECD and USEPA guidelines that were revised to marine species testing conditions. Marine species were chosen due to the fact that SAS is primarily used in off-shore drilling and therefore, marine species are the most relevant species. As shown in Table 6, SAS is virtually nontoxic to aquatic organisms.

Aquatic toxicity studies have been performed on marine fish, invertebrates, and algae, showing a low order of aquatic toxicity for SAS; fish appear to be the most sensitive species with 72 and 96 hour LC_{50} data showing toxicity at 1,672 mg/L.

Table 6. Results for Ecotoxicity Endpoints

Ecotoxicity Endpoints				
Test	**SAS**	**Sulfonic Acids, Petroleum Salts (Sur. #1)**	**Napthenic Acid (Sur. #2)**	**Asphalt (Su. #3)**
Acute/	24- and 48-hr LC_{50}	96-hr LL_0 =	96-hr TLm =	LC_{50} >1000

Ecotoxicity Endpoints				
Test	**SAS**	**Sulfonic Acids, Petroleum Salts (Sur. #1)**	**Napthenic Acid (Sur. #2)**	**Asphalt (Su. #3)**
Prolonged Toxicity to Fish	>1,800 mg/L, and 72- and 96-hr LC_{50} = 1,672 mg/L[4, S]	10,000 mg/L[5,C]	16.3 mg/L (ppm) [6,B] 96-hr LC50 ~ 5 mg/L[6,G]	mg/L (structural analogs C15 and greater)[7,O]
Acute Toxicity to Aquatic Invertebrates (*Daphnia*)	96-hr LC_{50} = 420,000 ppm[1,M]	ND	ND	LC_{50} >1000 mg/L (structural analogs C15 and greater) [7, D]
Acute Toxicity to Aquatic Plants (Algae)	NOEC = 1.0 grams per liter (g/L)[3,K] EC_{50} = 4.0 g/L[3,K]	ND	ND	LC_{50} >1000 mg/L (structural analogs C15 and greater) [7,P]
Other	Not toxic[2,N] 96-hr LC_{50} = 155,000 ppm (liquid phase bioassay) and 205,000 ppm (suspended particulate phase bioassay)[2,A]	ND	ND	ND

[1]Laboratory Technology, Inc., 1994.
[2]Marine Bioassay Laboratories, 1982.
[3]TNO, 1991a.
[4]Chemex Environmental International Limited, 2002.
[5]ACC, 2001. WAF (Water accommodated fraction static nonrenewal test.)
[6]API, 2003c.
[7]API, 2003d.
ND = No Data Available
[A]*Acanthomysis sculpta* (shrimp-like Mysids)
[B]*Brachydanio rerio* (Zebra Fish)
[C]*Cyprinodon variegatus* (Sheepshead Minnow)
[D]*Daphnia magna*
[G]*Gasterosteus aculeatus* (Three-spine Stickleback)
[K]*Skeletonema costatum*
[M]*Mysidopsis bahia* (Mysid shrimp)
[N]*Macoma nasuta* (Mollusca)
[O]*Oncorhynchus mykiss*
[P]*Selenastrum capricornutum*
[S]*Scophthalmus maximus* (Turbot)

Summary: Adequate aquatic toxicity data are available for SAS; no additional testing is proposed for the USEPA HPV Challenge Program (see Table 6 and IUCLID document).

VII. MAMMALIAN TOXICITY

Overall, available mammalian toxicity data on SAS, and its structural surrogates that represent many of the SAS functional groups and encompass the most toxicologically significant SAS constituents, indicate a low order of toxicity. SAS has only been tested for acute toxicity via the oral route, where results are of a similar order of magnitude for both SAS and all of the structural surrogates included in this test plan. This helps reinforce the suitability of using these surrogates as benchmarks for SAS. Acute dermal and inhalation toxicity results for the various structural surrogates likewise demonstrate a low order of toxicity for this class of materials. Repeated dose studies were identified for all three surrogates (dermal and inhalation studies for Surrogate #1, Ca salts of Petroleum-derived Sulfonic Acids; an oral study for Surrogate #2, Naphthenic Acids; and dermal and inhalation studies for Surrogate #3, Asphalts). In general, results indicated a low order of repeated dose toxicity by the dermal and inhalation routes, however, liver effects in the repeated dose oral toxicity study on Naphthenic Acid indicated that the liver may be a target organ. Neither *in vitro* nor *in vivo* genetic toxicity studies were identified for SAS. However, *in vitro* studies (both gene mutation and chromosomal aberration studies) were negative for all of the structural surrogates, with the exception of the Asphalts (Surrogate #3) where studies demonstrated that whole asphalts are nonmutagenic or are weakly mutagenic and that fume condensates are mutagenic with the severity of the effect correlating with the temperature under which the fumes are generated (API, 2003b). *In vivo* genetic toxicity studies were identified for Surrogates #1 (Ca salts of Petroleum-derived Sulfonic Acids) and #3 (Asphalts). While the Sulfonic Acids were found not to be genotoxic, conflicting results were reported for the Asphalts. No reproductive or developmental studies were identified for SAS or any of its structural surrogates.

Table 7. Results for Mammalian Toxicity Endpoints

Mammalian Toxicity Endpoints				
Test	**SAS**	**Sulfonic Acids, Petroleum Salts (Sur. #1)**	**Napthenic Acid (Sur. #2)**	**Asphalts (Sur. #3)**
Acute Oral	>5,000 milligrams per kilogram (mg/kg) bw (rat – m and f)[1, 2]	Na Salt: LD_{50}>5,000 mg/kg (rat).[3] Ca Salt: LD_{50}>5,000 mg/kg (rat).[3] Ba Salt: LD_{50}>2,000 mg/kg (rat).[3]	1. LD_{50} = 5,880 mg/kg (rat).[5] 2. LD_{50} = 3,000 mg/kg (rat) – fraction from crude kerosene acids[5] and	LD_{50} > 5,000 mg/kg bw (rat).[6]

Mammalian Toxicity Endpoints				
Test	**SAS**	**Sulfonic Acids, Petroleum Salts (Sur. #1)**	**Napthenic Acid (Sur. #2)**	**Asphalts (Sur. #3)**
			LD_{50} = 5,200 mg/kg (rat) – fraction from mixed crude oils.[5] 3. High dose effects (300 mg/kg bw): decreased food consumption; lethargy and mild ataxia; signif. inc. in relative organ weights of ovaries and spleen (female) and testes and heart (male); eosinophilic pericholangitis, and brain hemorrhage.[5]	
Acute Inhalation	ND	Na Salt: LC_0 > 1.9 mg/L (rat).[3,4]	ND	LC_{50} >94.4 mg/m^3 (rat).[6]
Acute Dermal	ND	Na Salt: LD_{50}>2,000 mg/kg (rabbit).[3] Ca Salt: LD_{50}>5,000 mg/kg (rabbit).[3]	LD_{50} = 3160 mg/kg (rabbit).[5]	LD_{50} >2,000 mg/kg bw (rabbit).[6]
Repeat Dose – Oral	ND	ND	NOAEL and LOAEL not provided, see description in Section B below.[5]	ND
Repeat Dose – Dermal	ND	Ca Salt: NOAEL = 1,000 mg/kg/day (highest dose tested) (rat – 28 day).[3]	ND	Rabbit Study – NOAEL and/or LOAEL not provided, see description in Section B below.
Repeat Dose – Inhale.	ND	Ca Salt: NOAEL = 49.5 milligrams per cubic meter (mg/$m^{3)}$ (rat – 28 day).[3]	ND	NOAEL = 28.17 mg/m^3 (rat).[6,7]
Genetic (*In vitro*) Gene Mutation	ND	Ca Salt: Bacterial Reverse Mutation Assay – not mutagenic – genotoxicity NOEL = 5,000 micrograms per plate (µg/plate).[3] Ca Salt: Mouse Lymphoma Mutagenicity Screen – not mutagenic.[3]	Negative (Ca and Na salts) – with and without activiation.[5]	Whole asphalts are nonmutagenic or weakly mutagenic, fume condensates are mutagenic (severity correlates with temperature under which fumes are generated).[7]

Mammalian Toxicity Endpoints				
Test	**SAS**	**Sulfonic Acids, Petroleum Salts (Sur. #1)**	**Napthenic Acid (Sur. #2)**	**Asphalts (Sur. #3)**
Genetic (*In vitro*) Chrom. Aberr.	ND	Ca Salt: CHO Cell Chromosomal Aberration Assay – not genotoxic.[3]	Negative (Na salt)[5] (Na salt was positive in sister chromatid exchange assay).[5]	Roofing asphalt fumes caused a dose-related increase in micronucleus formation in Chinese Hamster lung fibroblasts.[7] Three paving asphalt fume condensates were negative in an unspecified chromosome aberration assay.[7]
Genetic – *In vivo*	ND	Ca Salt: Mouse Micronucleus Assay – not genotoxic – genotoxicity NOEL = 2,000 mg/kg.[3]	ND	1. Two negative oral chromosome aberration studies on vacuum residuum samples.[7] 2. Increased micronucleus formation in bone marrow erythrocytes.[7] 3. Positive dermal and intratracheal instillation DNA adduct tests.[7]
Repro-duction/ Develop-mental Screen	ND	ND	ND	ND

[1]Hazleton Laboratories American, Inc., 1985a.
[2]Hazleton Laboratories American, Inc., 1985b.
[3]ACC, 2001.
[4]LC_0 = no mortality observed at the highest concentration tested.
[5]API, 2003c.
[6]API, 2003d.
[7]API, 2003b.
ND = No Data Available

Summary: Sufficient acute toxicity data are available for SAS and/or its surrogates. No further acute toxicity testing is proposed. Repeated dose studies were identified for all three surrogates (dermal and inhalation studies for Surrogate #1, Ca salts of Petroleum-derived Sulfonic Acids; an oral study for Naphthenic Acids; and dermal and inhalation studies for Surrogate #3, Asphalt). While multiple repeated dose toxicity studies are available, which encompass the alkylaryl, naphthenic, and asphalt functional groups, these data do not entirely address this endpoint for SAS. In addition, no reproductive or developmental studies were identified for SAS or any of its structural surrogates. To fulfill the repeated dose, reproductive, and developmental toxicity endpoints, a 28-day combined, repeated dose and reproductive/developmental toxicity screening study (OECD Guideline 422) is proposed. Genotoxicity data exist for all three structural surrogates. However, no specific genotoxicity data is available for SAS. CPChem proposes to conduct an AMES Test (OECD 471) to further support use of surrogate data presented in this test plan.

(See Table 7 and IUCLID Documents.)

A. Acute Toxicity

SAS demonstrated a low order of toxicity via the oral route of exposure (LD_{50} > 5,000 mg/kg body weight). Consistent results were seen for the three structural surrogates: the Na, Ca, and Ba salts of Petroleum-derived Sulfonic Acids (Surrogate #1); the Naphthenic Acids (Surrogate #2); and the Asphalts (Surrogate #3), as demonstrated in Table 7 above. In addition, acute dermal toxicity data for the various structural surrogates indicate that SAS would be expected to have a very low order of acute toxicity via this route as well (LD_{50} of 2,000 to 5,000 mg/kg in rabbits for Surrogate #1 [Na and Ca salts of Petroleum-derived Sulfonic Acids] and Surrogate #3 [Asphalts]). Limited data exist regarding the acute inhalation toxicity of this class of material. No acute inhalation toxicity data were identified for SAS, however an LC_0 of 1.9 mg/L (the maximum attainable concentration) was reported in rats exposed to Na salts of Petroleum-derived Sulfonic Acid and an LC_{50} of 9.4 mg/m^3 was reported for asphalt condensate fumes. Due to the low vapor pressure of SAS, exposure via the respiratory route is unlikely. As a result, the low order to toxicity demonstrated by the asphalt condensate fumes is expected to be a conservative benchmark for SAS.

Summary: These studies fulfill the HPV requirements for the acute toxicity endpoint; no additional testing is proposed for the USEPA HPV Challenge Program.

B. Repeated Dose Toxicity

No repeated dose toxicity data were identified for SAS. However, both a dermal and inhalation repeated dose study were identified for Surrogate #1 – Ca salts of Petroleum-derived Sulfonic Acids. In a 28-day dermal repeated dose study following OECD Guideline 410, a NOAEL of 1,000 mg/kg was established (highest dose

tested) and no signs of overt systemic toxicity were demonstrated. In a 28-day inhalation repeated dose study following OECD Guideline 412, a NOAEL of 49.5 mg/m^3 was established based on the slight, dose-related increase observed in the severity of microscopic pulmonary findings and increased lung weights.

A 90-day oral subchronic study in rats was identified for Surrogate #2, Naphthenic Acid (in aqueous solution, derived from Athabasca sands tailings). While neither a NOAEL or an LOAEL were reported, the following results were provided:

- Significant effects in the high dose group (60 mg/kg bw) included decreased food consumption immediately following dosing; severe, clonic seizures lasting 20 seconds in 25% of dosed animals (observed after day 40) – after which all animals, except one that died, resumed normal activity; lower mean body weight throughout the exposure period; increased relative organ weights in the liver, kidney, and brain; reduction in plasma cholesterol on days 45 and 91 (41 and 43%); increase in amylase activity on day 45 and 91 (33 and 30%); less pronounced differences in total protein concentration (increased) and albumin/globulin ratio (decreased); and five out of 12 rats with increased glycogen storage. Results indicate that the liver is likely a target organ.
- Significant effects in mid-dose group (6 mg/kg bw) included severe, clonic seizures lasting 20 sec in 17% of dosed animals (observed after day 40) – after which all animals, except one that died, resumed normal activity; and three out of 12 rats with increased glycogen accumulation.
- Significant effects in low-dose group (0.6 mg/kg bw) were minimal with only two out of 12 rats exhibiting increased glycogen accumulation.

Two 28-day dermal repeated dose toxicity studies in male and female New Zealand white rabbits were identified for Asphalt (Surrogate #3). In both studies, the rabbits were treated with 200, 1,000, and 2,000 mg/kg vacuum residuum samples API 81-13 and API 81-14 undiluted and occluded, once a day, three times a week for 4 weeks (API, 2003b). While neither a NOAEL nor a LOAEL were reported for either study, the following results were provided:

- Treatment-related clinical signs in animals that survived to day 28 (two animals died and two were sacrificed moribund during the study but none of these were considered to be compound-related) included thin appearance, decreased food intake, flaking skin, and wheezing.
- Edema was recorded in all groups except controls throughout the study; severity ranged from very slight to slight. Erythema could not be scored at most daily intervals because the test material could not be removed from the skin and therefore obscured the test site.
- At 2,000 mg/kg, a treatment-related suppression in body weight gain was recorded for the high dose male groups. Rabbits appeared thin, experienced decreased body weight gain, and decreased food intake. Significant differences included absolute kidney weight in males (-16%), absolute/

relative right adrenal weight in males (+ 86/133%), absolute pituitary weight in females (+63%), and relative spleen weight in females (+50%).

- At 1,000 mg/kg, significant differences were reported in male kidney weights (-14%).
- Flaking skin, acanthotic dermatitis, and hyperkeratosis were seen in males given 2,000 mg/kg API 81-13, and in both sexes, API 81-14 also produced wart-like lesions and white discharge at the treated site.
- No treatment-related trends in any of the hematological or clinical chemical parameters that were measured were reported.
- No systemic toxicity was reported.

A 90-day subchronic inhalation toxicity study following OECD Guideline 413 was also identified for Asphalt (Surrogate #3). In this study, male and female Wistar rats were exposed (nose-only) to asphalt fume condensate collected over a paving asphalt tank. Target concentrations were 0, 4, 20, and 100 mg/m^3. Actual mean concentrations measured by IR according to BIA (Germany) guideline #6305 and corrected for aromatic content (Ekström et al., 2001 in API, 2003), were 5.53, 28.17, and 149.17 mg/m^3 total hydrocarbon of bitumen fumes. The following results were provided:

- At 149.17 mg/m3, male rats exhibited statistically significant lower body weights with a concurrent decrease in food consumption, and female rats had slightly lower body weights than controls.
- Histopathological changes were observed in the nasal and paranasal cavities in both sexes.
- Broncho-alveolar lavage demonstrated a statistically significant increase in mean cell concentration, lactate dehydrogenase levels, and alpha glutamyl transferase levels in high dose female rats; effects in high dose males were similar but less pronounced.
- The NOAEL for this study was 28.17 mg/m^3 (API, 2003b).

Summary: While multiple repeated dose toxicity studies are available for SAS surrogates encompassing the alkylaryl, naphthenic, and asphalt functional groups, these data presented do not entirely address this endpoint for SAS. A 28-day combined, repeated dose, and reproductive/developmental toxicity screening study (OECD Guideline 422) is proposed.

C. Genetic Toxicity/Mutagenicity

1. *In Vitro* Gene Mutation and Chromosomal Aberration Studies

No studies were identified for SAS. However, gene mutation tests conducted on Ca salts of Petroleum-derived Sulfonic Acids (Surrogate #1) and the Ca and Na salts of Naphthenic Acid (Surrogate #2) consistently resulted in negative results using the Ames Test. Surrogate #1 was also tested in the mouse lymphoma mutagenicity screen test and was found not to be mutagenic. *In vitro* chromosomal aberration test results were identified as well for Surrogates #1 and

#3. Ca salts of Petroleum-derived Sulfonic Acids (Surrogate #1) were found to be "not genotoxic" in the CHO cell chromosomal aberration assay. Likewise, a study on the Na salt of Naphthenic Acid (Surrogate #2) was also found to be negative in a chromosomal aberration test conducted by the National Toxicology Program (API, 2003a).

Ambiguous results were reported for Asphalt (Surrogate #3). In general, *in vitro* studies demonstrated that whole asphalts are nonmutagenic or are weakly mutagenic, and that fume condensates are mutagenic with the severity of the effect correlated with the temperature under which fumes are generated. Asphalt fumes generated at > 232°C (>450°F) exhibited moderate mutagenicity while asphalt fumes generated at 163 C (325°F) exhibited lower mutagencitiy (API, 2003b).

In contrast to asphalt, SAS does not emit fumes during its normal intended use (typical surface temperatures during drilling operations ranges between 100-150° F (37-66°C)). CPChem proposes an initial genotoxicity screen using Ames Test (OECD 471) for 3 reasons. 1) similar sensitivities of detecting a positive genotoxic insult have been described for the *in vitro* Ames Test versus the rodent micronucleus assay; furthermore animal use is minimized, 2) a potential exists for osmotic/ionic disruptions to occur in the *in vitro* chromosome aberration test caused by the sodium salt of SAS; hence increasing the possibility of false positive results (2004 Mid America Toxicology Course Syllabus; oral communication with D.J. Brusick), and 3) as an initial screen to strengthen SAR data (currently negative genotoxicity with the exception of contradicting asphalt fume data) presented in this test plan and/or future surrogate data being generated through the US HPV Challenge Program.

Summary: CPChem proposes to conduct an Ames Test (OECD 471).

2. *In Vivo* Genetic Toxicity/Mutagenicity

No studies were identified for SAS. However, Ca salts of Petroleum-derived Sulfonic Acids (Surrogate #1) were found to be "not genotoxic" in the mouse micronucleus assay. However, *in vivo* studies for Asphalt (Surrogate #3) presented conflicting results. *In vivo* genetic toxicity data included two negative oral chromosome aberration studies on vacuum residuum samples, a micronucleus test in which asphalt fume condensate instilled intratracheally induced increased micronucleus formation in bone marrow erythrocytes, and positive dermal and intratracheal instillation DNA adduct tests. Sponsors of the Asphalt Category indicate that the conflicting results in the *in vivo* cytogenetic assays presented above should be resolved by an ongoing micronucleus evaluation being conducted in rats exposed to bitumen fumes in an ongoing lifetime inhalation study (API, 2003b).

Summary: No Testing proposed. Based on results from the *in vitro* Ames Test mentioned above, CPChem may find that further testing is required using the *in vivo* mouse micronucleus test (OECD 474).

D. Reproductive/Developmental Toxicity

No reproductive or developmental toxicity studies were identified for SAS or any of its structural surrogates (with the exception of a reproductive toxicity study for Surrogate #3 [Naphthenic Acid], which was not of sufficient data quality and was in abstract form only) (API, 2003a). However, considering the high molecular weight, limited bioavailability, and minimal observed general toxicity of SAS, SAS is unlikely to cause developmental or reproductive effects.

Summary: Given that no data are available for the reproductive and developmental toxicity endpoints and to provide definitive data for SAS, testing is proposed following OECD Guideline 422 "Combined Repeated Dose Toxicity Study with the Reproduction/Developmental Toxicity Screening Test".

VIII. CONCLUSIONS

As summarized below, adequate data (i.e., Klimisch rating 1 and 2) are available for several endpoints. However, testing is proposed for the following endpoints:

- water solubility;
- repeated dose toxicity;
- *in vitro* genetic toxicity/mutation;
- reproduction toxicity; and
- developmental toxicity.

PHYSICOCHEMICAL DATA. EPIWIN data show representative structures to SAS and its surrogates will have high melting point ranges (>349.84 °C), boiling point ranges > 900 °C, and very low vapor pressures. SAS has a water soluble fraction and is water dispersible by design; therefore a water solubility continuum will occur in which SAS fractions may be soluble across a range from ppm to zero. Importantly, the high degree of sulfonation significantly reduces octanol solubility such that SAS constituents will have a very low Kow, where Kow values can range from 2 to 7, which suggests that there is low cause for concern for bioaccumulation. The weight of evidence supports that adequate data are available for the physicochemical endpoints, with the exception of water solubility. For the USEPA HPV Challenge Program, testing is proposed following OECD Guideline 105 to fulfill the water solubility endpoint.

ENVIRONMENTAL FATE. Values for SAS photodegradation and atmospheric oxidation were calculated based upon representative chemical structures using EPIWIN; no further tested is proposed. Components in SAS do not undergo hydrolysis as they do not contain hydrolysable components. As a result, no further

hydrolysis testing is warranted for SAS. Results from the Level III fugacity modeling of representative structures indicate that releases of SAS to water would remain in water and releases to soil would remain in soil while releases to air would partition primarily to soil. Further fugacity modeling is not warranted for the USEPA HPV Challenge Program. Lastly, SAS has been tested in a Ready Biodegradation test and was not readily biodegradable (3 to 6% in 28 days and 0% in 56 days). No further biodegradability testing is proposed. The weight of evidence in this test plan supports that no further environmental fate testing is necessary to meet HPV SIDS endpoints relating to environmental fate; therefore, no additional testing is proposed for the USEPA HPV Challenge Program.

ACUTE AQUATIC TOXICITY. Acute fish, daphnid, and algal endpoints for SAS are fulfilled with valid studies that were conducted consistent with relevant OECD and USEPA guidelines. No further testing is proposed.

ACUTE MAMMALIAN TOXICITY. Available mammalian toxicity data on SAS (and its structural surrogates that represent the most toxicologically significant SAS constituents) indicate a low order of toxicity. SAS has only been tested for acute toxicity via the oral route, where results are of a similar order of magnitude for both SAS and all of the structural surrogates included in this test plan. Acute dermal and inhalation toxicity results for the various structural surrogates likewise demonstrate a low order of toxicity for this class of materials. No further acute toxicity testing is proposed.

REPEATED DOSE TOXICITY. While multiple repeated dose toxicity studies are available for SAS surrogates encompassing the alkyl aryl, naphthenic, and asphalt functional groups, these data do not entirely address this endpoint for SAS. A 28-day combined, repeated dose, and reproductive/developmental toxicity screening study (OECD Guideline 422) is proposed.

GENETIC TOXICITY. Genotoxicity data exist for all three structural surrogates, Petroleum-derived Sulfonic Acids, Naphthenic Acids, and Asphalts. However, no specific genotoxicity data is available for SAS. CPChem proposes to conduct an AMES Test (OECD 471) to support use of surrogate data presented in this test plan. Based on the evaluation of these data, further genotoxicity tests may be required, ie, *in vivo* micronucleus test (OECD 474).

REPRODUCTIVE AND DEVELOPMENTAL TOXICITY. Neither SAS nor any of its structural surrogates have been tested for reproductive and developmental toxicity. To provide definitive data for SAS, testing is proposed following OECD Guideline 422 "Combined Repeated Dose Toxicity Study with the Reproduction/Developmental Toxicity Screening Test".

X. REFERENCES

American Chemistry Council (ACC). 2001. High Production Volume (HPV) Challenge Program Test Plan for Petroleum Additive Alkaryl Sulfonate Category, Prepared by The American Chemistry Council Petroleum Additives Panel, Health, Environmental, and Regulatory Task Group. Submitted to the US EPA October 2001 and posted November 30, 2001.

American Petroleum Institute (API), Petroleum HPV Testing Group. 2003a. Test Plan for Reclaimed Substances: Streams Containing Naphthenic Acids, Phenolics, Disulfides, Acids or Caustics. Submitted to the US EPA December, 2003 and posted January 20, 2004.

API, Petroleum HPV Testing Group. 2003b. Test Plan for Asphalt Category. Submitted to the US EPA December 15, 2003 and posted January 20, 2004.

API, Petroleum HPV Testing Group. 2003c. Robust Summary of Information on Reclaimed Substances: Naphthenic Acid. Submitted to the US EPA December 15, 2003 and posted January 20, 2004.

API, Petroleum HPV Testing Group. 2003d. IUCLID Data Set. Submitted to the US EPA December 15, 2003 and posted January 20, 2004.

Atkinson, R. 1990. Gas-phase tropospheric chemistry of organic compounds: A review. *Atmos. Environ.*, 24A: 1-41. In API, 2003b.

Boethling, R.S. and J. V. Nabholz. 1996. Environmental Assessment of Polymers under the U.S. Toxic Substances Control Act. In *Ecological Assessment of Polymers – Strategies for Product Stewardship and Regulatory Programs*, ed. J.D. Hamilton and R. Sutcliff.

Chemex Environmental International Limited. 2002. The toxicity to Turbot (*Scophthalmus maximus*) of Soltex Additive, Report for Drilling Specialties Company, Chemex reference ENV6103/05022. Cambridge, England.

Chemex Environmental International Limited. 2003. The Bioaccumulation Potential of Sulphonated Asphalt Additive, Report for Drilling Specialties Company, Chemex reference ENV6222/100202.

Commission of the European Communities (EC). 2003. Proposal for a Regulation of the European Parliament and of the Council Concerning the Registration, Evaluation, Authorisation and Restrictions of Chemicals (REACH), COM(2003) 644, Volume II, Brussels, 29 October 2003.

Ekström, L.G., A.J. Kriech, C. Bowen, S. Johnson, and D. Breuer. 2001. International studies to compare methods for personal sampling of bitumen fumes. J. Environ. Monitoring 3: 439-445. In API, 2003b.

Harris, J.C. 1982. Rate of Hydrolysis, *Handbook of Chemical Property Estimation Methods*. W.L. Lyman, W.F. Reehl, and D.H. Rosenblastt, eds. McGraw-Hill Book Co., New York, NY. In API, 2003a.

Hayashi, M., MacGregor, J.T., Gatehouse, D.G. et al. 2000. In Vivo Rodent Erythrocyte Micronucleus Assay. II. Some Aspects of Protocol Design Including Repeated Treatments, Integration With Toxicity Testing, and Automated Scoring. *Environ. Mol. Mutgen.* 35: 234-252.

IARC (International Agency for Research on Cancer). 1985. Polynuclear Aromatic Compounds. Part 4, Bitumins, Coal tars, and Derived Products, Shale oils and Soots. Vol. 35 in the IARC Monographs on the Evaluation of the Carciogenic Risk of Chemicals to Humans, pp. 39-81. Lyon, France. In API, 2003b.

Klimisch, H.J., E. Andreae, and U. Tillmann. 1997. A systematic approach for evaluating the quality of experimental and ecotoxicological data. *Reg. Tox. and Pharm.* 25: 1-5.

Laboratory Technology, Inc. 1994. 96 Hour Range Finder Acute Toxicity Test of Drilling Fluid Suspended Particulate Phase, Based on Permit# GMG290000, Prepared for Drilling Specialties Company, Lab Technology Control # DS019. Kenner, Louisiana.

Marine Bioassay Laboratories. 1982. Drilling Mud Bioassay, Soltex, *Acanthomysis sculpta* and *Macoma nasuta*, Prepared for IMCO Services and Drilling Specialties Company. Watsonville, California.

Organisation for Economic Co-operation and Development (OECD) Secretariat. 2002. *Manual for Investigation of HPV Chemicals* (November 2002).

Phillips Petroleum Company. 1985. Research and Development Report 10035-85, June 17, 1985.

Puzinauskas, V.P. and L.W. Corbett. 1978. Differences between petroleum asphalt, coal tar, pitch and road tar. Research Report 78-1. Asphalt Institute, College Park MD. In API, 2003b.

TNO Environmental and Energy Research (TNO). 1991a. Effect of a 1% Soltex® Solution (262-100-3) on the Growth of the Marine Alga *Skeletonema costatum*, (ISO/DIS 10253) TNO Study no. IMW-91-0021-01. Delft, the Netherlands.

TNO. 1991b. The Biodegradability of the Product Soltex® Shale Inhibitor in Seawater According to a Proposed EC Test Guideline (Closed Bottle Test), TNO Study no. IMW-91-0072-01. Delft, the Netherlands.

TNO. 1993. The Biodegradability of the Product 3.5% Bentonite Slurry with 1% Soltex® (262:100-2) in Seawater According to a Proposed EC Test Guideline (Closed Bottle Test), TNO Study no. IMW-91-0073-01. Delft, the Netherlands.

TNO. 1997. Determination of the Partition Coefficient (n-octanol/water), HPLC method, of Soltex® Shale Inhibitor, Final Report, Prepared for Drilling Specialties Company, TNO Study no. BIO-96-0115-01 (460784). Breda, the Netherlands.

USEPA. 1999a. Draft Determining the Adequacy of Existing Data. USEPA, Office of Pollution Prevention and Toxics. Washington, D.C.

USEPA. 1999b. The Use of Structure-Activity Relationships (SAR) in the High Production Volume Chemicals Challenge Program. USEPA, Office of Pollution Prevention and Toxics. Washington, D.C.

USEPA, Office of Pollution Prevention and Toxics and Syracuse Research Corporation. 2000. EPI Suite v 3.10 (April, 2001).

Witherspoon, P.A. 1962. Colloidal nature of petroleum. *Trans. NY Acad. Sci.* 25: 344-361.

Appendix II

DATA QUALITY ASSESSMENT

Available environmental, ecotoxicity, and mammalian toxicity studies were reviewed and assessed for reliability according to standards specified by Klimisch et al., (1997), as recommended by the USEPA (1999a) and the OECD (OECD, 2002). The following reliability classification (Klimisch rating) has been applied to each study assessed:

- *1 = Reliable without Restriction* – Includes studies that comply with USEPA- and/or OECD-accepted testing guidelines and were conducted using Good Laboratory Practices (GLPs) and for which test parameters are complete and well documented;

- *2 = Reliable with Restriction* – Includes studies that were conducted according to national/international testing guidance and are well documented. May include studies that were conducted prior to establishment of testing standards or GLPs but meet the test parameters and data documentation of subsequent guidance; also includes studies with test parameters that are well documented and scientifically valid but vary slightly from current testing guidance. Also included in this category were physical-chemical property data obtained from reference handbooks, as well as environmental endpoint values obtained from an accepted method of estimation (e.g., USEPA's EPIWIN estimation program);

- *3 = Not Reliable* – Includes studies in which there are interferences in either the study design or results that provide scientific uncertainty or in which documentation is insufficient; and,

- *4 = Not Assignable* – This designation is used in this dossier for studies that appear scientifically valid but for which insufficient information is available to adequately judge robustness.

Those studies receiving a Klimisch rating of 1 or 2 are considered adequate to support data assessment needs in this dossier. Those key studies selected for inclusion are considered typical of the endpoint responses observed in other studies of a similar nature and design that were identified during our search of the literature.

Appendix II

LINKS TO SURROGATE TEST PLANS AND ROBUST SUMMARIES

SURROGATE 1:

Alkaryl Sulfonates
Sulfonic Acids, petroleum salts
CAS Number 68783-96-0 (sodium salt)
CAS Number 61789-86-4 (calcium salt)
CAS Number 61790-48-5 (barium salt)
http://www.epa.gov/chemrtk/alklsulf/c13206tc.htm
Sponsor: American Chemistry Council

SURROGATE 2:

Reclaimed Substances Category
Napthenic Acids, Petroleum, crude
CAS Number 64754-89-8
http://www.epa.gov/chemrtk/resbscat/c14906tc.htm
Sponsor: The American Petroleum Institute Petroleum HPV Testing Group

SURROGATE 3:

Asphalt Category
http://www.epa.gov/chemrtk/asphlcat/c14901tc.htm
Sponsor: The American Petroleum Institute Petroleum HPV Testing Group

201-15220

I U C L I D

Data Set

Existing Chemical	:	ID: 68201-32-1
CAS No.	:	68201-32-1
EINECS Name	:	Asphalt, sulfonated, sodium salt
EINECS No.	:	269-212-0

Producer Related Part

Company	:	Chevron Phillips Chemical Company LP
Creation date	:	09.01.2004

Substance Related Part

Company	:	Chevron Phillips Chemical Company LP
Creation date	:	09.01.2004
Memo	:	
Printing date	:	30.01.2004
Revision date	:	
Date of last Update	:	30.01.2004
Number of Pages	:	30
Chapter (profile)	:	Chapter: 1, 2, 3, 4, 5, 7
Reliability (profile)	:	Reliability: without reliability, 1, 2, 3, 4
Flags (profile)	:	Flags: without flag, confidential, non confidential, WGK (DE), TA-Luft (DE), Material Safety Dataset, Risk Assessment, Directive 67/548/EEC, SIDS

1.0.1 OECD AND COMPANY INFORMATION

Type	:	other
Name	:	Chevron Phillips Chemical Company LP
Partner	:	
Date	:	
Street	:	10001 Six Pines Drive
Town	:	77380 The Woodlands, TX
Country	:	United States
Phone	:	
Telefax	:	
Telex	:	
Cedex	:	

09.01.2004

2.1 MELTING POINT

2.2 BOILING POINT

2.4 VAPOUR PRESSURE

2.5 PARTITION COEFFICIENT

Log pow	:	< 0 - 6.2 at 22° C
Method		OECD Guide-line 117 "Partition Coefficient (n-octanol/water), HPLC Method"
Year	:	1997
GLP	:	yes
Test substance	:	other TS

Remark : The following interpretation of the results were provided by Ambiorn Hanstveil (TNO Nutrition and Food Research Institute, Toxicology Division) to the Drilling Specialties Company:

- Four components with a log Pow </= 3, i.e. peak numbers 1 to 4, are considered to have no potential for bioaccumulation.
- One component with a log Pow = 3.2, i.e. peak number 5, is a limit case. Depending on its molecular weight, it will have a limited potential for bioaccumulation or none at all.
- Two components with a log Pow > 6.2, i.e. peak numbers 7 and 8, have extreme long retention times compared to the reference substances. These peaks are therefore considered to represent components with very high log Pow values, that have no potential for bioaccumulation.

Result : The partition coefficient (n-octanol/water) range determined for Soltex Shale Inhibitor by HPLC was:

Range log Pow: < 0, 1.1, 3.2, and > 6.2

It was noted that Soltex Shale Inhibitor did not completely dissolve in methanol. From the observation of eight major peaks in HPLC chromatograms, the partition coefficient (log Pow) for the fraction of Soltex Shale Inhibitor soluble in methanol, was calculated to range from < 0 to > 6.2, i.e. lower than zero and higher than the highest partition coefficient of the reference components, which in this study was 4,4'-DDT. In addition to the major eight peaks, minor peaks could be observed in the chromatogram of the test substance after elution of the last reference component. The partition coefficients for these peaks can also be regarded as > 6.2.

RESULTS FOR SOLTEX SHALE INHIBITOR:
Results are presented in the following format: peak / tR (min) / k / log k / calculated log Pow:

peak 1 / 1.27 / -0.56 / n.a. / < 0
peak 2 / 1.46 / -0.49 / n.a. / < 0
peak 3 / 1.98 / -0.31 / n.a. / < 0
peak 4 / 2.77 / -0.03 / n.a. / < 0
peak 5 / 3.04 / 0.06 / -1.201 / 1.1

peak 6 / 4.21 / 0.47 / -0.326 / 3.2
peak 7 / 164.69 / 56.58 / n.a. / > 6.2
peak 8 / 222.30 / 76.73 / n.a. / > 6.2

Explanation:
k: capacity factor = (tR - t0) / t0
tR: retention time of the reference or test substance
t0: dead-time (i.e. retention time of formamide)
n.a.: not applicable, not within range of reference substances

RESULTS FOR REFERENCE SUBSTANCES:
Results are presented in the following format: reference material / tR (min) / k / log k / log Pow / log Pow back-calculated:

phenol / 3.03 / 0.06 / -1.23 / 1.5 / 1.1
phenol / 3.05 / 0.07 / -1.18 / 1.5 / 1.2
toluene / 4.20 / 0.47 / -0.33 / 2.7 / 3.2
toluene / 4.19 / 0.47 / -0.33 / 2.7 / 3.2
naphthalene / 4.98 / 0.74 / -0.13 / 3.6 / 3.7
naphthalene / 4.92 / 0.72 / -0.14 / 3.6 / 3.7
biphenyl / 6.33 / 1.21 / 0.08 / 4.0 / 4.2
biphenyl / 6.11 / 1.14 / 0.06 / 4.0 / 4.1
phenanthrene / 10.01 / 2.50 / 0.40 / 4.5 / 5.0
phenanthrene / 9.50 / 2.32 / 0.37 / 4.5 / 4.9
4,4'-DDT / 14.69 / 4.14 / 0.62 / 6.2 / 5.5
4,4'-DDT / 13.47 / 3.71 / 0.57 / 6.2 / 5.4

Explanation:
k: capacity factor = (tR - t0) / to
tR: retention time of the reference or test substance
to: dead-time i.e. retention time of formamide: 2.86 minutes (average of 2.88 and 2.84 minutes)

Source : Phillips Petroleum Company, Determination of the Partition Coefficient (n-octanol/water), HPLC method, of Soltex Shale Inhibitor. Study performed by BCO Analytical Services B.V, Breda, The Netherlands for Drilling Specialties Company, Bartlesville, Oklahoma.

Test condition : TEST AND REFERENCE MATERIALS
- Test substance was a product sample of Soltex Shale Inhibitor (Drilling Specialties Company, Bartlesville, Oklahoma).
- Chemical composition: sulphonated asphaltenes
- (Hot) water solubility: high (determined by Soxhlet extraction with water)
- References substances were:
----phenol, 99.5%, CASN 108-95-2
----toluene, 99%, CASN 108-88-3
----naphthalene, 100%, CASN 91-20-3
----biphenyl, 99%, CASN 92-52-4
----phenanthrene, 98%, CASN 85-01-8
----4,4'-DDT, 99%, CASN 50-29-3
- Preparation of test solutions: A solution of Soltex Shale Inhibitor was prepared by accurately weighing 11 mg and dissolving in 100 ml methanol (99.5%, CASN 67-56-1). The fraction soluble in methanol was further used in the study. The test solution was used directly for preparation of UV/VIS spectrum and concentrated ten times (by evaporation) for HPLC analysis.
- Preparation of reference substance and formamide solutions (a mixture was prepared form the following solutions by combining 200 ul of the solutions of phenol, toluene, naphthalene and 4,4'-DDT and 20 ul of the solutions of biphenyl and phenanthrene with 100 ul of the formamide solution):
----phenol: 11 mg dissolved in 10 ml methanol
----toluene: 149 mg dissolved in 10 ml methanol

----naphthalene: 10 mg dissolved in 10 ml methanol
----biphenyl: 11 mg dissolved in 1.0 ml methanol
----phenanthrene: 10 mg dissolved in 10 ml methanol
----4,4'-DDT: 9 mg dissolved in 10 ml methanol
----formamide: 110 mg dissolved in 10 ml methanol

EQUIPMENT AND REAGENTS
- The HPLC was equipped with a Vydac 201 TPB (C18, 25 cm id. 4.6 mm) column.
- Elution: isocratic
- Mobile phase: methanol / Suprapur water 75:25 (v/v)
- Detector: UV 210 or 254 nm
- Flow rate: 1.0 ml/minute
- Temperature: 22 +/- 0.2 deg C
- Injection volume: 20 ul

METHOD OF ANALYSIS
- Determination of dead time: determined from the duplicate measurements of retention time for formamide.
- References and calibration: A calibration graph was prepared for the references in order to correlate the measured capacity factor k with the Pow of the test substance. The mixture of the reference substances was injected in duplicate onto the HPLC column and the resulting retention times were measured. The calibration curve was prepared by plotting log k versus log Pow for the reference compounds.
- Log Pow values of the reference substances were:
----phenol = 1.5
----toluene = 2.7
----naphthalene = 3.6
----biphenyl = 4.0
----phenanthrene = 4.5
----4,4'-DDT = 6.2

CONDUCT OF THE TEST
- HPLC analysis of the test substance was performed and the range of retention times of the detectable components was determined. In addition, the retention times of peaks that could be distinguished within this range were measured.
- The applicability of the use of the wavelengths (210 or 254 nm) of the UV detector was verified by recording the UV/VIS absorption spectrum of the test solution of 0.11 g/l Soltex Shale Inhibitor in methanol (using blank methanol as reference).
- The spectrum was recorded using a Perkin-Elmer lambda 2 UV/VIS spectrometer and quartz cuvette (pathlength 1 cm).

CALCULATIONS
- Calibration curves were prepared by linear regression analysis with spreadsheets in Lotus 1-2-3 version 2.2.
- The HPLC analysis of the test substance resolved in a band of analytical signals (some clearly visible as peaks) on the HPLC chromatograms. From the first and last detectable signal of the test substance, the upper and lower limits of log Pow were determined. In addition, the log Pow was calculated for the clearly distinguishable peaks.
- Results (partition coefficient of the test substances) of peaks within the range of retention times of reference substances, were calculated by interpolation of the calculated capacity factor k on the calibration curve.
- When peaks were outside the range of retention times observed for the reference substances, the log Pow values were set at either below zero or greater than 6.2.

Test substance : Asphalt, sulfonated, sodium salt, CAS Number 68201-32-1. XXXXXXXX XXXXXXX. Trade Name: Soltex Shale Inhibitor.

Reliability : (1) valid without restriction
Flag : Critical study for SIDS endpoint
30.01.2004 (1)

Log pow : < 0 at ° C
Method OECD Guide-line 117 "Partition Coefficient (n-octanol/water), HPLC Method"

Year : 2003
GLP : yes
Test substance : other TS

Result : Three peaks were detected with UV and none with RI. The log Pow values were all < 0.0.

These results show all peaks have log Pow values less than 3.0 and are consistent with a material which has little tendency to accumulate in the environment.

Results are for the water soluble portion of the sample only and are provided in the following format:
Peak / Retention time (mins) Run 1 / Retention time (mins) Run 2 / Peak Area (%) Run 1 / Peak Area (%) Run 2 / k Run 1 / k Run 2 / log k Run 1 / log k Run 2 / Log Pow Run 1 / Log Pow Run 2

Peak 1 / 2.044 / 2.247 / 85.38 / 86.11 / -0.25 / -0.18 / * / * / * / *
Peak 2 / 2.336 / 2.344 / 10.40 / 9.89 / -0.15 / -0.15 / * / * / * / *
Peak 3 / 2.605 / 2.614 / 4.22 / 4.01 / -0.05 / -0.05 / * / * / * / *

* Unable to calculate as k is negative (tR < t0)
t0 = 2.744

CALIBRATION DATA:
Results are presented in the following format: Reference material / Retention time (mins) / k / log k / log Pow from the literature:

Benzene / 4.983 / 0.82 / -0.09 / 2.1
Toluene / 6.385 / 1.33 / 0.12 / 2.7
Ethyl benzene / 8.125 / 1.96 / 0.29 / 3.2
Propyl benzene / 11.168 / 3.07 / 0.49 / 3.7
Butyl benzene / 16.067 / 4.86 / 0.69 / 4.6
DDT / 35.547 / 11.96 / 1.08 / 6.2

t0 = 2.744

Source : Phillips Petroleum Company, The Bioaccumulation Potential of Sulphonated Asphalt Additive - Report for Drilling Specialties Company. Study performed by Chemex Environmental International Limited, Cambridge, England for Drilling Specialties Company, Bartlesville, Oklahoma.

Test condition : TEST SUBSTANCE: Sulphonated Asphalt Additive supplied by Drilling Specialties Company, purity not known.

REFERENCE SUBSTANCES:
- Benzene: log Pow (from literature) 2.1, 99.7%, 0.0016 ul injected.
- Toluene: log Pow (from literature) 2.7, 99.95%, 0.0016 ul injected.
- Ethyl benzene: log Pow (from literature) 3.2, 99.0%, 0.0016 ul injected.
- Propyl benzene: log Pow (from literature) 3.7, 98.0%, 0.0016 ul injected.
- Butyl benzene: log Pow (from literature) 4.6, 99+%, 0.0016 ul injected.
- DDT: log Pow (from literature) 6.2, 98.0%, 6.86 ug injected.

- Thiourea: 99.0%, 0.15 ug injected.

SAMPLE PREPARATION
- 0.1 g of test material was dispersed in 10 ml of pH 8 aqueous buffer and syringe filtered (0.45 um) to remove un-dissolved sample. 7.5 ml of methanol was added to 2.5 ml of the filtrate and this was injected in duplicate (0.025 g in 10 ml). The quantities injected were 0.05 mg.

INSTRUMENTATION
- Chromatography System: Perkin Elmer Quaternary System
- HPLC gradient pump: Perkin Elmer Series 200
- UV detector: Perkin Elmer 785A UV/VIS @ 210 nm (1.0V/AU)
- RI detector: Perkin Elmer LC-25
- Interface box: 900 Series and 600 Link Series
- Software: PE Nelson Turbochrom Workstation
- Analyical column: Hypersil, 5 um, C18, 250 by 4.6 mm

CONDITIONS
- Mobile phase: 75:25 methanol: 0.02M phosphate buffer (pH 8.0)
- Flow rate: 1 ml/min
- Injection volume: 20 ul (standard) and 20 ul (sample and blank)
- The system dead time (t0) is the average retention time of a non-retained material. the dead time is taken as the retention time of the thiourea peak (or the solvent front when the thiourea and solvent coelute).

Test substance : Asphalt, sulfonated, sodium salt, CAS Number 68201-32-1. XXXXXXXXX XXXXX.

Reliability : (1) valid without restriction
Flag : Critical study for SIDS endpoint
30.01.2004 (3)

2.6.1 WATER SOLUBILITY

3.1.1 PHOTODEGRADATION

3.1.2 STABILITY IN WATER

3.3.1 TRANSPORT BETWEEN ENVIRONMENTAL COMPARTMENTS

Type	:	fugacity model level III
Media	:	other: air-water-soil-sediment
Air (level I)	:	
Water (level I)	:	
Soil (level I)	:	
Biota (level II / III)	:	
Soil (level II / III)	:	
Method	:	other: EPIWIN v 3.10
Year	:	2004

Method : Used EPIWIN v 3.10. The following physical properties were used as the model input parameters:

Chem Name: Three representative structures, C26 H45 O3 S1 Na1; C26 H43 O9 S3 Na3; and C40 H61 O15 S5 Na5
Molecular Wt: 460.7; 664.78; 1057.2
Henry's LC (atm-m3/mole): 6.07E-7; 1.79E-20; 9.33E-34
Vapor Press (mm Hg): 6.02E-18; 3.9E-23; 1.75E-33
Liquid VP (mm Hg): 5.51E-15; 6.36E-20; 2.86E-30
Melting Pt (deg C): 324; 350; 350
Log Kow: 6.78; 2.32; 4.05
Soil Koc: 2.47E+6; 85.7; 4.6E+3

Result : Results are provided in the following format:
Compartment / 100% to Air/ 100% to Water / 100% to Soil/ Equally to Each Compartment

(C26 H45 O3 S Na)
Air / 5.33% / 0.000131% / 0.00% / 0.444%
Water / 2.47% / 12.3% / 0.00161% / 8.2%
Soil / 74.7% / 0.00183% / 100% / 33.1%
Sediment / 17.5% / 87.7% / 0.0114% / 58.2%

Persistence when distributed equally to each compartment = 643 hr
(Emissions [kg/hr] = 1000 to air, 1000 to water, 1000 to soil, and 0 to sediment).

(C26 H43 O9 S3 Na3)
Air / 0.0055% / 0.00% / 0.00% / 0.35%
Water / 10.3% / 99.5% / 5.66% / 31.5%
Soil / 89.6% / 0.00% / 94.3% / 68.0%
Sediment / 0.0557% / 0.537% / 0.0306% / 0.17%

Persistence when distributed equally to each compartment = 707 hr
(Emissions [kg/hr] = 1000 to air, 1000 to water, 1000 to soil, and 0 to sediment).

(C40 H61 O15 S5 Na5)
Air / 0.00% / 0.00% / 0.00% / 0.00246%
Water / 3.55% / 82.3% / 0.156% / 15.3%
Soil / 95.7% / 0.00% / 99.8% / 81.4%
Sediment / 0.763% / 17.7% / 0.0334% / 3.29%

Persistence when distributed equally to each compartment = 1.6E+3 hr (Emissions [kg/hr] = 1000 to air, 1000 to water, 1000 to soil, and 0 to sediment).

Source : EPI Suite v 3.10.

Test substance : Three representative structures: C26 H45 O3 S1 Na1; C26 H43 O9 S3 Na3; and C40 H61 O15 S5 Na5

Reliability : (2) valid with restrictions
Flag : Critical study for SIDS endpoint
30.01.2004 (16)

3.5 BIODEGRADATION

Type : aerobic
Inoculum :
Concentration : 3 mg/l related to Test substance

Contact time : 56 day
Degradation : 0 - 3 % after 56 day
Result : under test conditions no biodegradation observed
Deg. Product :
Method : other: EC Guideline "Biotic degradation in seawater: Closed Bottle Method"
Year : 1993
GLP : yes
Test substance : other TS

Result : No oxygen consumption was found which could be attributed to the biological degradation of Soltex.

The results showed the expected rapid degradation of acetate (complete within seven days). The measured oxygen consumption of acetate was 0.66 mg O2/mg after seven days and compared well with the theoretical oxygen demand of 0.68 mg O2/mg.

The calculated oxygen consumption of acetate in the presence of the test substance was slightly lower than found with acetate alone, indicating that Soltex had a slightly inhibiting effect on the acetate degradation.

The oxygen consumption due to the acetate in presence of the 3.5% bentonite slurry was similar to that of acetate and Soltex, confirming that the effect recorded above was probably caused by the bentonite slurry.

Soltex was not degraded in the presence of acetate.

Source : Phillips Petroleum Company, The Biodegradability of the Product 3.5% Bentonite Slurry with 1% Soltex (262:100-2) in Seawater According to a Proposed EC Test Guideline (Closed Bottle Test). Study performed by TNO Environmental and Energy Research, Delft, The Netherlands for Drilling Specialties Company, Bartlesville, Oklahoma.

Test condition : TEST SUBSTANCE
- 3.5% bentonite slurry with 1% Soltex (262:100-2), a dark brown liquid.

NATURAL SEAWATER
- A sample of natural seawater was taken from the Eastern Scheldt (Jacobahaven) on August 28, 1991.
- Water temperature was 18.5 deg C and the salinity was 33.3%.
- The sample was taken 2.5 meters above the bottom about one hour after low tide and was aerated until the test started.
- TOC of the seawater was found to be <0.8 mg C/L.
- Total chlorophyll and phaeophytine content: 4.30 - 4.95 mg/m3.
- Content of NO3, NH4+, and PO4-3 was < 15, 0.16, and <0.10 mg/L respectively.
- 1 ml of each of the following nutrient stock solutions were added per litre of natural seawter prior to use in order to prevent nutrient limitation:
----Stock solution a per 1000 ml milli-Q-water: 8.50 g KH2PO4, 21.75 g K2HPO4, 50.14 g Na2HPO4.7H2O, 1.70 g HN4Cl
----Stock solution b per 1000 ml milli-Q-water: 22.5 g MgSO4.7H2O
----Stock solution c per 1000 ml milli-Q-water: 36.4 g CaCl2.2H2O
----Stock solution d per 1000 ml milli-Q-water: 0.15 g FeCl3

TEST METHOD
- A concentration of 2 mg test substance per litre usually allows the determination of 95% degradation. However, on the basis of TNO's experience in testing this chemical substance in seawater, one high concentration was tested.
- A test substance concentration equivalent to about 3 mg/L of Soltex was used.
- A test concentration of 301 mg/L (262:100-2, corresponding to 3.0 mg/L Soltex) was prepared by adding 1.8038 g of test substance to 6.0 litre of natural seawater.
- In order to check the toxicity of the test substance, a test concentration of 299.5 mg/L was prepared by adding 1.2580 g of test substance to 4.2 litre of natural seawater containing 4.0 mg/L sodium acetate.
- In order to check the bacterial activity of the seawater itself additional bottles were prepared containing only 4 mg/L soidum acetate as carbon source.
- Each test solution was distributed over thirteen bottles. In addition, nineteen bottles were prepared with natural seawater only to serve as control for the oxygen consumption by the seawater itself.
- Triplicate BOD bottles were prepared for each treatment. The initial oxygen concentration was measured with an oxygen electrode in one bottle of each treatment.
- The other bottles were then closed and incubated at about 20 deg C in the dark.
- The O2 concentrations were measured again after 7, 14, 21, and 28 days (all treatments) and 42 and 56 days (reference and test substance). A separate set of bottles was sacrificed for each measurement.

CALCULATION OF RESULTS
- The oxygen demand in each test bottle after 7, 14, 21, 28, 42 and 56 days was calculated by subtracting the oxygen concentration measured at that time from that measured at the start of the test.
- The Biochemical Oxygen Demand (BOD) due to the test or control substances at each time was calculated (in mg O2/L) by subtracting the oxygen demand in the relevant inoculum control or other control bottle from that in the bottle under consideration; these crude values were then converted to values per mg substance.
- The degradation was expressed as BOD as percentage of the COD of the test substance.

Test substance : 3.5% bentonite slurry with 1% Soltex (Asphalt, sulfonated, sodium salt, CAS Number 68201-32-1. (XXXXXXXXXXXX).

Reliability	:	(1) valid without restriction
Flag	:	Critical study for SIDS endpoint
30.01.2004		(12)
Type	:	aerobic
Inoculum	:	
Concentration	:	3.8mg/l related to Test substance
Contact time	:	28 day
Degradation	:	3 - 6 % after 28 day
Result	:	other: low biodegradability in seawater.
Deg. Product	:	
Method	:	other: EC Guideline "Biotic degradation in seawater: Closed Bottle Method"
Year	:	1991
GLP	:	yes
Test substance	:	other TS

Result :

The oxygen consumption due to the test substance was low, representing at most 3-6% of the Chemical Oxygen Demand (COD). It was concluded that Soltex Shale Inhibitor has a low biodegradability in seawater.

The results showed the expected rapid degradation of the acetate control substance (complete within 14 days). The measured oxygen consumption of acetate was 0.66 mg O2/mg after 14 days and compared well with the theoretical oxygen demand (TOD) of 0.68 mg O2/mg.

Source :

Phillips Petroleum Company, The Biodegradability of the Product Soltex Shale Inhibitor in Seawater According to a Proposed EC Test Guideline (Closed Bottle Test). Study performed by TNO Environmental and Energy Research, Delft, The Netherlands for Drilling Specialties Company, Bartlesville, Oklahoma.

Test condition :

TEST SUBSTANCE
- Soltex Shale Inhibitor, batch no. 90-7101, a dark brown to black granular material.

NATURAL SEAWATER
- A sample of natural seawater was taken from the Eastern Scheldt (Jacobahaven) on April 9, 1991.
- Water temperature was 9 deg C and the salinity was 3.26%.
- The sample was taken 2.5 meters above the bottom about half an hour before high tide and was aerated until the test started.
- Total chlorophyll and phaeophytine content: 4.8 - 5.9 mg/m3.
- Content of NO3, NH4+, and PO4-3 was 2.4, 0.06, and 0.08 mg/L respectively.
- 27 ml of each of the following nutrient stock solutions were added to 27 litres of natural seawter prior to use in order to prevent nutrient limitation:
----Stock solution a per 1000 ml milli-Q-water: 3.50 g KH2PO4, 21.75 g K2HPO4, 50.14 g Na2HPO4.7H2O, 1.70 g HN4Cl
----Stock solution b per 1000 ml milli-Q-water: 22.5 g MgSO4.7H2O
----Stock solution c per 1000 ml milli-Q-water: 36.4 g CaCl2.3H2O
----Stock solution d per 1000 ml milli-Q-water: 0.15 g FeCl3

TEST METHOD
- A concentration of 2 mg test substance per litre usually allows the determination of 95% degradation. It was not possible to disperse the test substance in water or an organic solvent and a higher concentration had to be tested.
- About 1 mg dry test substance was added to each bottle of about 295 ml (the volume of the individual bottles varied between 294.9 and 295.3 ml) resulting in a mean test substance "concentration" of about 4 mg/L.
- Two series of 12 bottles were prepared, one containing the test substance

alone, and one toxicity control series containing 4 gm/L sodium acetate in addition to the test substance.
- Two bottles without test substance were prepared for determination of the initial oxygen concentration.
- In order to check the bacterial activity of the seawater itself, 13 additional bottles were prepared containing only 4 mg/L sodium acetate as carbon source.
- In addition, 13 bottles with seawater without any additions were prepared to serve as a control series for the oxygen consumption by the seawater itself.
- After the oxygen concentration had been determined in one bottle of each treatment using an ocygen electrode, the other twelve bottles were closed and incubated at about 20 deg C in the dark.
- After 7, 14, 21, and 28 days, three bottles of each treatment were sacrificed for determination of the oxygen concentration.

CALCULATION OF RESULTS
- The oxygen demand in each test bottle after 7, 14, 21, and 28 days was calculated by subtracting the oxygen concentration measured at that time from that measured at the start of the test.
- The Biochemical Oxygen Demand (BOD) due to the test or control substances at each time was calculated (in mg O2/L) by subtracting the oxygen demand in the relevant inoculum control or other control bottle from that in the bottle under consideration; these crude values were then converted to values per mg substance.
- The degradation was expressed as BOD as percentage of the COD of the test substance.

Test substance : Asphalt, sulfonated, sodium salt, CAS Number 68201-32-1. XXXXXXXX XXXXX. Trade Name: Soltex Shale Inhibitor.

Reliability : (1) valid without restriction
Flag : Critical study for SIDS endpoint
30.01.2004 (13)

4.1 ACUTE/PROLONGED TOXICITY TO FISH

Type	:	semistatic
Species	:	other: Scophthalmus maximus
Exposure period	:	96 hour(s)
Unit	:	mg/l
Analytical monitoring	:	
LC50	:	= 1672
LC50 24h	:	> 1800
LC50 48h	:	> 1800
Method	:	OECD Guide-line 203 "Fish, Acute Toxicity Test"
Year	:	2002
GLP	:	yes
Test substance	:	other TS

Method : Based on OECD 203 and modified to marine conditions.

Result :

- 96-hour LC50 = 1672 mg/l (1194 to 2342 95% confidence limit interval)
- 24-hour and 48-hour LC50 > 1800 mg/l.
- The highest no-observed (lethal) effect concentration (NOEC) was estimated as 1000 mg/l.
- The lowest observed (lethal) effect concentration (LOEC) was 1800 mg/l.
- The lowest concentration giving 100% mortality could not be determined as there was only 57.1% mortality in the highest concentration.
- A mortality of 0% was observed in the control tank at the end of the test period.
- The test substance nominal concentrations were prepared as "water-accommodated fractions" according to the OSPAR guidelines, but significant amounts of test material remained suspended after the nominal settling period. The solution was filtered using a 63 um sieve to remove the suspended material and the subsequent filtrate used for the test. However, in test concentrations it was observed that some material sedimented out of solution at 24 hours onwards. This increased in quantity with increase in concentration and time.
- There was no chemical analytical confirmation of the actual dissolved concentrations. The dissolved concentrations were likely to be lower than the nominal concentrations as Soltex Additive was poorly soluble in water.

RAW DATA
- Cummulative percent mortality results are presented in the following format: Exposure period (hours) / control / 560 mg/l / 1000 mg/l / 1800 mg/l

0 / 0 / 0 / 0 / 0
24 / 0 / 0 / 0 / 0
48 / 0 / 0 / 0 / 14.3
72 / 0 / 0 / 0 / 57.1
96 / 0 / 0 / 0 / 57.1

Source : Phillips Petroleum Company, The Toxicity to Turbot (Scophthalmus maximus) of Soltex Additive - Report for Drilling Specialties Company. Study performed by Chemex Environmental International Limited, Cambridge, England for Drilling Specialties Company, Bartlesville, Oklahoma.

Test condition : TEST SPECIES
- Turbot (Scophthalmus maximus)
- Acclimation period: 6 June to 1 July 2002
- Acclimation conditions: Temperature: 13.5 to 14.5 deg C; Dissolved oxygen: >95% ASV
Mean length: 43.5 mm
Mean weight: 2.13 g

DILUTION WATER:
- The stocks of animals were maintained, and the tests performed, in standardised artificial seawater using Tropic Marin artificial sea salt.
- The measured salinity of the seawater used was 31 to 35 g/l sodium chloride.

TEST METHODS AND CONDITIONS
- A nominal 1000 mg/l solution of Soltex Additive was prepared in dilution water, shaken vigorously and allowed to stand for four hours.
- For each nominal concentration the required amount of homogenised sample was added to 12 litres of dilution water, mixed for 20-24 hours and then allowed to separate for four hours. The solution was filtered using a 63 um sieve, the filtrate was used for the test.
- A preliminary study had identified the 96 hour LC50 as being > 1000 mg/l and therefore definitive test concentrations were prepared as 0 (Control), 560, 1000, and 1800 mg/l.
- Volumes of 10 litres of test solution were prepared in aquaria. A control vessel of 10 litres dilution water was prepared.
- Seven turbot were placed in each of the test and control vessels.
- The pH value (to 0.1), dissolved oxygen (to 1% ASV), and temperature (to 0.5 deg C) were measured on each test and control solution immediately prior to initiating the test.
- The test and control solutions were replaced at 48 hours, the remaining live animals being transferred to freshly prepared test solutions.
- The test parameters were measured before and after each change of test solution, and observations of mortality were made daily.
- The test vessels were maintained at 15 +/- 1.5 deg C, with a light cycle of 16 hours light and 8 hours dark.

STATISTICS
- Cumulative mortalities were calculated for each test concentration and the control. LC50 values were estimated and 95% confidence limits calculated using ToxCalc version 5.0 "Comprehensive Toxicity Data Analysis and Database Software."

Test substance : Asphalt, sulfonated, sodium salt, CAS Number 68201-32-1 (XXXXXXXXX XXXXX). Trade Name: Soltex Additive.

Reliability : (2) valid with restrictions
Flag : Critical study for SIDS endpoint
30.01.2004 (2) (10) (14)

4.2 ACUTE TOXICITY TO AQUATIC INVERTEBRATES

Type	:	static
Species	:	Mysidopsis bahia (Crustacea)
Exposure period	:	96 hour(s)
Unit	:	mg/l
Analytical monitoring	:	no data
EC50	:	= 420000
Method	:	other: EPA 40 CFR Part 435
Year	:	1994

GLP : no data
Test substance : other TS

Result : 96-hour LC50 was 420,000 ppm (95% confidence interval of 368,000 ppm to 481,000 ppm).

The 96- hour LC50 for the standard reference toxicant (sodium lauryl sulfate) was 8.5 ppm, with a 95% confidence interval of 8.0 ppm to 9.1 ppm.

RAW DATA:
Results presented in the following format: Concentration (%) / Number exposed / Mortalities

0 / 20 / 0
1 / 20 / 0
3 / 20 / 0
5 / 20 / 0
10 / 20 / 0
25 / 20 / 0
50 / 20 / 15
100 / 20 / 20

Spearman-Karber Estimates:
- LC50: 42.04
- 95% lower confidence: 36.76
- 95% upper confidence: 48.08

Source : Phillips Petroleum Company, 96 Hour Range Finder Acute Toxicity Test of Drilling Fluid Suspended Particulate Phase - Based on Permit #: GMG290000. Study performed by Laboratory Technology, Inc., Kenner, Louisiana for Drilling Specialties Company, Bartlesville, Oklahoma.

Test condition : MATERIALS AND METHODS
- Based on those suggested by the EPA (40 CFR Part 435; 8/26/85).
- All equipment was washed with detergent, rinsed with tap water, acetone, deionized water, soaked in a 10% HCL bath, rinsed with tap water and finally deionized water.

Artificial Seawater Preparation: Made by mixing a commercial brand of synthetic sea salts (Hawaiian Marine Mix) with deionized water. The seawater was prepared at a salinity of 20 +/- 2 ppt and stored in a opaque drum with continuous aeration. Water was "seasoned" for several days before use and filtered through a 1.0 micron filter.

Organism Acquisition and Maintenance: Mysid shrimp (Mysidopsis bahia) were raised and maintained at 25 +/- 1 deg C and 20 +/- 2 ppt salinity. During maintenance and testing, mysids were fed approximately 50 brine shrimp nauplii per mysid daily. Test organisms were 4 to 6 days old.

TEST MEDIA PREPARATION:
- A one-half gallon sample of drilling fluid from Drilling Specialties Company was provided and stored at 4 deg C. The drilling fluid had a pH of 6.05 and did not emit a foul odor. The sample was thoroughly mixed for 30 minutes prior to use.
- The suspended particulate phase (SPP) was prepared by mixing the mud sample and artificial seawater in a 1 to 9 ratio in 2-L large-mouth Erlenmeyer flask.
- Mud/seawater slurry mixed for 5 minutes.
- pH of the slurry was measured and adjusted, if necessary, to within 0.2 units of the seawater by adding diluted hydrochloric acid while stirring.
- Slurry was allowed to settle for one hour.

- Supernatant (SPP) was decanted and SPP was mixed for another 5 minutes while the pH and dissolved oxygen were measured and adjusted if necessary.
- If the dissolved oxygen was less than 4.9 ppm, the SPP was aerated to at least 4.9 ppm which is 65 percent of saturation. Maximum aeration time was 5 minutes.
- The filterable and non-filterable residue of each SPP was measured according to the methods listed in the ASTM.

EXPERIMENTAL CONDITIONS
SPP test was conducted at 20 +/- 2 ppt salinity and 20 +/- 1 deg C. Dissolved oxygen, temperature, salinity, and pH were measured at 0, 24, 48, 72, and 96 hours.
- Test media was aerated during the entire test at an estimated rate of 50-144 cubic centimeters per minute.
- Light/dark cycle was maintained at 14L/10D during maintenance and testing.

EXPERIMENTAL PROCEDURE
- Nytex cups were used to confine the mysids in each test concentration. Cups were positioned in 8 inch Carolina Culture dishes which contained 1 liter of the test solution.
- 20 organisms were exposed to each test concentration of the prepared SPP, the control, and the standard reference toxicant.
- Organisms were selected, transferred and assigned to treatments, containers, and positions according to a modified randomization procedure as described in the EPA 40 CFR part 435.
- All live organisms were counted at 0, 24, 48, 72, and 96 hours in those dishes where turbidity and color did not preclude observation.

Test substance : Drilling fluid from Drilling Specialties Company containing Asphalt, sulfonated, sodium salt, CAS Number 68201-32-1. (XXXXXXXXXXXX).

Reliability : (2) valid with restrictions
Flag : Critical study for SIDS endpoint
30.01.2004 (8) (15)

Type : static
Species : other: Acanthomysis sculpta
Exposure period : 96 hour(s)
Unit : mg/l
Analytical monitoring :
EC50 Suspended Particulate Phase : = 205000
EC50 Liquid Phase : = 155000
Method : other: EPA Region 2 Drilling Mud Bioassay
Year : 1982
GLP : no data
Test substance : other TS

Method : Drilling Mud bioassay Test Procedures to be Employed Under EPA, Region 2, Offshore Exploratory Drilling Permits, Annexes I, II, and III. Procedures employed in bioassay testing were generally in accordance with those developed by the Mid-Atlantic Joint Industry Bioassay Program.

Result : The 96-hour LC50 values for Acanthomysis sculpta were 155,000 ppm in the Liquid Phase bioassay and 205,000 ppm for the Suspended Particulate Phase bioassay.

RAW DATA
Liquid Phase Bioassay:
- Number of Survivors -- Results are presented in the following format:

Test Medium Concentration (ppm v/v) / Replicate / 0 hr / 4 hr / 8 hr / 24 hr / 48 hr / 72 hr / 96 hr

1,000,000 / 1 / 10 / 10 / 7 / 4 / 0 / 0 / 0
1,000,000 / 2 / 10 / 10 / 8 / 3 / 0 / 0 / 0
1,000,000 / 3 / 10 / 9 / 9 / 2 / 1 / 0 / 0
1,000,000 / 4 / 10 / 10 / 9 / 4 / 0 / 0 / 0
1,000,000 / 5 / 10 / 10 / 7 / 4 / 0 / 0 / 0

500,000 / 1 / 10 / 10 / 10 / 7 / 0 / 0 / 0
500,000 / 2 / 10 / 10 / 10 / 9 / 0 / 0 / 0
500,000 / 3 / 10 / 10 / 10 / 9 / 0 / 0 / 0
500,000 / 4 / 10 / 10 / 10 / 6 / 0 / 0 / 0
500,000 / 5 / 10 / 10 / 10 / 8 / 8 / 0 / 0

200,000 / 1 / 10 / 10 / 10 / 10 / 9 / 8 / 7
200,000 / 2 / 10 / 10 / 10 / 8 / 8 / 6 / 5
200,000 / 3 / 10 / 10 / 10 / 8 / 8 / 6 / 5
200,000 / 4 / 10 / 10 / 10 / 9 / 9 / 5 / 4
200,000 / 5 / 10 / 10 / 10 / 10 / 7 / 3 / 3

100,000 / 1 / 10 / 10 / 10 / 9 / 9 / 9 / 9
100,000 / 2 / 10 / 10 / 10 / 8 / 8 / 7 / 7
100,000 / 3 / 10 / 10 / 10 / 10 / 10 / 10 / 9
100,000 / 4 / 10 / 10 / 10 / 10 / 10 / 9 / 9
100,000 / 5 / 10 / 10 / 10 / 9 / 9 / 8 / 8

50,000 / 1 / 10 / 10 / 10 / 10 / 10 / 10 / 10
50,000 / 2 / 10 / 10 / 10 / 10 / 9 / 8 / 8
50,000 / 3 / 10 / 10 / 10 / 10 / 10 / 10 / 10
50,000 / 4 / 10 / 10 / 10 / 9 / 9 / 8 / 8
50,000 / 5 / 10 / 10 / 10 / 10 / 10 / 10 / 10

0 (Control) / 1 / 10 / 10 / 10 / 10 / 10 / 9 / 9
0 (Control) / 2 / 10 / 10 / 10 / 10 / 10 / 10 / 10
0 (Control) / 3 / 10 / 10 / 10 / 10 / 10 / 10 / 10
0 (Control) / 4 / 10 / 10 / 10 / 9 / 9 / 9 / 9
0 (Control) / 5 / 10 / 10 / 10 / 10 / 10 / 10 / 10

Suspended Particulate Phase Bioassay:
- Number of Survivors results are presented in the following format: Test Medium Concentration (ppm v/v) / Replicate / 0 hr / 4 hr / 8 hr / 24 hr / 48 hr / 72 hr / 96 hr

1,000,000 / 1 / 10 / 10 / 9 / 7 / 0 / 0 / 0
1,000,000 / 2 / 10 / 10 / 9 / 8 / 1 / 0 / 0
1,000,000 / 3 / 10 / 10 / 10 / 7 / 0 / 0 / 0
1,000,000 / 4 / 10 / 10 / 9 / 6 / 0 / 0 / 0
1,000,000 / 5 / 10 / 10 / 8 / 7 / 0 / 0 / 0

500,000 / 1 / 10 / 10 / 10 / 7 / 4 / 2 / 2
500,000 / 2 / 10 / 10 / 10 / 7 / 7 / 4 / 4
500,000 / 3 / 10 / 10 / 10 / 8 / 6 / 4 / 3
500,000 / 4 / 10 / 10 / 10 / 7 / 6 / 4 / 2
500,000 / 5 / 10 / 10 / 10 / 5 / 5 / 5 / 2

200,000 / 1 / 10 / 10 / 10 / 10 / 9 / 7 / 7
200,000 / 2 / 10 / 10 / 10 / 9 / 8 / 8 / 8
200,000 / 3 / 10 / 10 / 10 / 9 / 8 / 8 / 8
200,000 / 4 / 10 / 10 / 10 / 7 / 7 / 7 / 6
200,000 / 5 / 10 / 10 / 10 / 8 / 8 / 4 / 4

100,000 / 1 / 10 / 10 / 10 / 9 / 9 / 9 / 9
100,000 / 2 / 10 / 10 / 10 / 7 / 7 / 6 / 6
100,000 / 3 / 10 / 10 / 10 / 10 / 10 / 7 / 7
100,000 / 4 / 10 / 10 / 10 / 9 / 9 / 8 / 8
100,000 / 5 / 10 / 10 / 10 / 10 / 10 / 10 / 9

50,000 / 1 / 10 / 10 / 10 / 10 / 10 / 10 / 10
50,000 / 2 / 10 / 10 / 10 / 10 / 10 / 10 / 10
50,000 / 3 / 10 / 10 / 10 / 8 / 8 / 7 / 7
50,000 / 4 / 10 / 10 / 10 / 8 / 8 / 8 / 8
50,000 / 5 / 10 / 10 / 10 / 9 / 9 / 9 / 8

0 (Control) / 1 / 10 / 10 / 10 / 10 / 10 / 9 / 9
0 (Control) / 2 / 10 / 10 / 10 / 10 / 10 / 10 / 10
0 (Control) / 3 / 10 / 10 / 10 / 10 / 10 / 10 / 10
0 (Control) / 4 / 10 / 10 / 10 / 9 / 9 / 9 / 9
0 (Control) / 5 / 10 / 10 / 10 / 10 / 10 / 10 / 10

Source : Phillips Petroleum Company, Drilling Mud Bioassay - Soltex - Acanthomysis sculpta and Macoma nasuta. Study performed by Marine Bioassay Laboratories, Watsonville, California for IMCO Services (Houston, Texas) and Drilling Specialties Company (Houston, Texas).

Test condition : LABORATORY FACILITIES
- Bioassay procedures conducted in MBL's marine laboratory located on the beach at Davenport Landing, California.
- Seawater system includes tandem intake lines extending 180 meters seaward from the beach and all cast-iron pumps delivering a flow of up to 2500 ppm each.
- Water is continuously supplied for use either unfiltered, sand-filtered, or sub-micron filtered, and can be heated or cooled to within 0.3 deg C of the desired temperature.
- 14-hour light/ 10-hour dark photoperiod during animal acclimation and testing periods.
- Test containers were wide-mouth glass jars (3.78 liters) containing 2 liters of test material.

TEST ORGANISMS
- Acanthomysis sculpta were collected by MBL personnel from kelp beds near Monterey, California and transported in aerated plastic buckets.
- Mysids were held for acclimation to test temperature and Davenport seawater for at least two days prior to testing.
- During acclimation and testing, mysids were fed brine shrimp nauplii.

TEST MATERIAL SAMPLING AND PREPARATION
- The drilling mud to be bioassayed was prepared and packed according to Region 2 procedures. Samples were stored at 2-4 deg C until preparation began.
- After preliminary pH testing and inspection, 22.7 liters of composited sample were transferred to a clean 190 liter polyethylene barrel and 90.8 liters of Davenport seawater were added.
- The pH of the resulting mud-seawater slurry was checked and found to be within 0.1 pH unit of ambient seawater.
- The mud-seawater slurry was mixed by vigorous aeration for 30 minutes.
- Following a one hour settling period the resulting elutriate (which required no centrifugation) was siphoned into clean buckets.
- The remaining sediment was reserved for use as the Solid Phase bioassay test material.
- Half the elutriate was filtered through a pre-washed 0.5 uM cartridge type acetate filter and retained as Liquid Phase test material. The unfiltered elutriate comprised the Suspended Particulate Phase test material.

BIOASSAY TEST PROCEDURES
- Liquid and Suspended Particulate Phase bioassays were conducted concurrently.
- Two liters of freshly prepared and appropriately diluted test material was added to each jar.
- Five replicates of five test concentrations and of control seawater were established and ten animals were used for each replicate.
- Survivors of the original ten animals per jar were recorded as test data at 4, 8, 24, 48, 72, and 96 hours after testing began.
- Dead animal were removed.
- Dissolved oxygen, temperature, salinity and pH were measured in each test container once daily.
- Mysids were fed once each day with 50-100 Artemia nauplii per mysid.

DATA ANALYSIS
- In order to facilitate calculation, LC50 values were obtained by computer regression analysis, modified to accommodate the probit (mortality) and logrithmic (elutriate concentration) scaled axes.

Test substance : Drilling mud from Drilling Specialties Company containing Asphalt, sulfonated, sodium salt, CAS Number 68201-32-1. (XXXXXXXXXXX). Trade Name: Soltex.

Reliability : (2) valid with restrictions
Flag : Critical study for SIDS endpoint
30.01.2004 (9)

4.3 TOXICITY TO AQUATIC PLANTS E.G. ALGAE

Species	:	Skeletonema costatum (Algae)
Endpoint	:	growth rate
Exposure period	:	95 hour(s)
Unit	:	g/l
Analytical monitoring	:	
NOEC	:	= 1
EC50	:	= 4
EC10	:	= .5
EC90	:	= 29.5
Method	:	other: ISO/DIS 10253
Year	:	1991
GLP	:	yes
Test substance	:	other TS

Result : The EC50 with respect to inoculum viability followed by logistic growth (EeC50) was found to be 4.0 g/L (95% confidence interval of 2.2 - 7.3 g/L). The corresponding EeC10 and EeC90 values were 0.5 and 29.5 g/L respectively.

The EC50 with respect to the area under the growth curve (EbC50) was found to be 8.3 g/L (in the range 3.2 - 10.0 g/L). The corresponding EbC10 and EbC90 values were 0.7 g/L (in the range 0.3 - 1.0 g/L) and > 10.0 g/L respectively.

The no-observed-effect-concentration (NOEC) was estimated to be 1.0 g/L.

The EbC and the EeC values were in the same range confirming the biomass dependent nature of the effects of the test substance.

Results were based on nominal concentrations of 1% Soltex Solution.

Microscopic examination of the cells at the start and end of the incubation period revealed no abnormalities; the chainlength (number of cells per particle) of the algal particles was, however, low in all cultures except that exposed to 3.28 g/L test substance.

RAW DATA
- Results of the model calculations for effect on inoculum viability followed by logistic growth (EeC50) -- Calculated Values (1E+3 particles/ml) where results are presented in the following format: Time (h) / 0 g/L 1% Soltex / 0.3 g/L 1% Soltex / 1.0 g/L 1% Soltex / 1.8 g/L 1% Soltex / 3.3 g/L 1% Soltex / 10 g/L 1% Soltex

0.0 hr / 0.7 / 0.7 / 0.6 / 0.5 / 0.4 / 0.2
22.5 hr / 6.6 / 6.2 / 5.4 / 4.6 / 3.7 / 1.8
47.0 hr / 61.2 / 58.3 / 51.4 / 45.4 / 37.1 / 19.3
68.0 hr / 213.0 / 208.4 / 196.4 / 184.6 / 165.7 / 108.8

- Mean area under the growth curve (A)
0 g/L 1% Soltex = 14332
0 g/L 1% Soltex = 13078
0.3 g/L 1% Soltex = 13307
1.0 g/L 1% Soltex = 11843
1.8 g/L 1% Soltex = 10932
3.3 g/L 1% Soltex = 9688
10 g/L 1% Soltex = 6308

- Percentage reduction in growth (IA)
0 g/L 1% Soltex = 0%
0 g/L 1% Soltex = 0%
0.3 g/L 1% Soltex = 3%
1.0 g/L 1% Soltex = 14%
1.8 g/L 1% Soltex = 20%
3.3 g/L 1% Soltex = 29%
10 g/L 1% Soltex = 54%

Source : Phillips Petroleum Company, Effect of a 1% Soltex Solution (262-100-3) on the Growth of the Marine Alga Skeletonema costatum (ISO/DIS 10253). Study performed by TNO Environmental and Energy Research, Delft, The Netherlands for Drilling Specialties Company, Bartlesville, Oklahoma.

Test condition : TEST SUBSTANCE:
- 1% Soltex Solution (262-100-3), a black liquid.
- Sample was stored at room temperature.
- Sample was stated to be soluble in water
- Sample prepared by sponsor as follows: "Using the Soxhlet Extraction procedure, dissolved 1.75 g Soltex in 175 ml tap water. The insoluble portion of Soltex was removed from the extraction thimble, and it was added to the Soltex solution."

TEST ORGANISM
- Marine alga Skeletonema costatum (ISTPM P4).
- A preculture of algae in the exponential growth phase was prepared as detailed in ISO/DIS 10253.

TEST MEDIUM
- Prepared in natural seawater with a salinity of approximately 32% and sterilized by micropore filtration.
- Stock solution 1: 3.2 mg/L K3PO4.H2O and 50.0 mg/L NaNO3
- Stock solution 2: 14.9 mg/L Na2SiO3.9H2O
- Stock solution 3: 140.0 ug/L C6H8O7Fe.3H2O; 605.0 ug/L MnCl2.4H2O; 150.0 ug/L ZnSO4.7H2O; 0.6 ug/L CuSO4.5H2O; 1.5 ug/L CoCl2.6H2O; 17.1 mg/L H3BO3; and 15.0 mg/L Na2EDTA.

- Stock solution 4: 25 ug/L Thiamin hydrochloride, 0.005 ug/L Biotin, and 0.05 ug/L B12.
- The medium as prepared by making 1 ml of stock solution 1, 0.52 ml of stock solution 2, 10 ml of stock solution 3, and 1 ml of stock solution 4 up to one litre with natural seawater.
- The pH was 8.0 +/- 0.2 after equilibration.

PREPARATION OF TEST SOLUTIONS
- Stock solutions prepared by dissolving 10, 97.6, and 1009.0 mg respectively in 1000 ml of algal medium (range-finding test) or 0.150, 0.52, 0.91, 1.64, and 5.01 g respectively in 500 ml of algal medium (growth inhibition test).
- The stock solutions for the range-finding test were used directly in the test.
- From the stock solutions prepared for the growth inhibition test, appropriate dilutions were prepared in algal medium to yield final concentrations of 0, 0.30, 1.0, 1.8, 3.3, and 10.0 g/L.

RANGE-FINDING TEST
- 2.6 ml of algal preculture containing 7.6E+4 particles/ml was added to a hundred ml of the appropriate solutions of the test substance and yielded a mean measured inoculum particle density in the control cultures of 2.2E+3 particles/ml.
- Test carried out in duplicate with two controls with algae only and a single background series containing test substance without algae.
- All flasks were incubated at 20 +/- 1 deg C and shaken (100 rpm) in an orbital shaker.
- Light intensity radiated by the fluorescent lamps was within the standard range of 60-120 umol/s/m2.
- After 3 days of incubation one sample was taken from each flask, and the number of particles per ml in the samples was determined with the aid of a Coulter Counter model TAII.

GROWTH INHIBITION TEST
- Test flasks, test solutions, and algal medium were prepared as detailed above.
- A suspension of algae in the algal medium containing 1E+5 cells/ml was prepared by dilution of a preculture containing 3.1E+5 particles/ml.
- Addition of 1.0 ml of this algal suspension to 100 ml of the appropriate solutions of the test substance in the test flasks yielded a mean measured inoculum particle density in the control cultures of 0.8E+3 particles/ml.
- All flasks were incubated at 20 +/- 1 deg C and shaken (100 rpm) in an orbital shaker.
- One sample was taken from each flask after 0, 22.5, 47, 68, and 95 hours, and the number of algal cells per ml in the samples was determined with the aid of a Coulter Counter model TAII.
- pH was measured at the start (medium without algae) and after 68 and 95 h in selected cultures. The pH of the algal medium at the start of the test was 7.8. The pH of the medium containing different test substance concentrations remained constant (pH 8.0 - 8.1) during the test. In the presence of algae, however, the pH was found to increase with algal cell density to pH 8.7 - 9.1 after 3 days and to pH 8.4 - 8.7 after 4 days.
- The morphology of the algae was examined visually with the aid of a microscope at the start and at the end of the test.

CALCULATION OF EC VALUES
- Algal particle density was obtained by subtraction of the number of particles in the background control series (without algae) from thenumber of particles in the test series. The mean values calculated were used for further calculations.
- The effect of a test substance on the growth of algae is expressed by quantities denoted as EC10, EC50, or EC90, i.e., the concentration of test

substance that reduced the growth rate, the yield or the viability of the inoculum cells by 10%, 50%, or 90% respectively.
- EC values with respect to the inoculum viability followed by logistic growth (EeC values), assuming an error proportional to the number of particles, were calculated by means of a parametric model developed by Kooijman et al. (1983). The values obtained in the last sampling period (95h) were omitted for model calculations because the S. costatum cell chains had broken, resulting in irregular particle counts.
- EC values with respect to the area under the growth curve (EbC values) were calculated by the method given in ISO/DIS 10253. The values were calculated by a linear interpolation of a plot of the percentage reduction in growth (IA) against the log concentration of the test substance.

DETERMINATION OF THE NOEC
- The "no-observed-effect-concentration" was estimated by visual comparison of both the measured and calculated growth curves of the treated algal suspensions with those of the controls.

Test substance : 1% Soltex Aqueous Solution (262-100-3). Asphalt, sulfonated, sodium salt, CAS Number 68201-32-1. XXXXXXXXXXX. Trade Name: Soltex
Reliability : (1) valid without restriction
Flag : Critical study for SIDS endpoint
30.01.2004 (6) (7) (11)

4.5.2 CHRONIC TOXICITY TO AQUATIC INVERTEBRATES

Species : other aquatic mollusc: Macoma nasuta
Endpoint : mortality
Exposure period : 10 day
Unit : mg/l
Analytical monitoring :
Method : other: EPA Region 2 Drilling Mud Bioassay
Year : 1982
GLP : no data
Test substance : other TS

Method : Drilling Mud bioassay Test Procedures to be Employed Under EPA, Region 2, Offshore Exploratory Drilling Permits, Annexes I, II, and III. Procedures employed in bioassay testing were generally in accordance with those developed by the Mid-Atlantic Joint Industry Bioassay Program.

Result : There was only a single mortality of Macoma nasuta in five experimental tanks (mean percent survival = 99%). This result was not statistically different compared to its control and it was concluded that SOLTEX drilling mud is not lethally toxic to Macoma nasuta.

RAW DATA
Results are presented in the following format: Replicate / Percent Survival at Day 10 in Control / Percent Survival at Day 10 in Soltex

1 / 100% / 95%
2 / 100% / 100%
3 / 100% / 100%
4 / 100% / 100%
5 / 100% / 100%

Mean / 100% / 99%
Variance (s2) / 0.0 / 5.0

Source : Phillips Petroleum Company, Drilling Mud Bioassay - Soltex -

Acanthomysis sculpta and Macoma nasuta. Study performed by Marine Bioassay Laboratories, Watsonville, California for IMCO Services (Houston, Texas) and Drilling Specialties Company (Houston, Texas).

Test condition : LABORATORY FACILITIES
- Bioassay procedures conducted in MBL's marine laboratory located on the beach at Davenport Landing, California.
- Seawater system includes tandem intake lines extending 180 meters seaward from the beach and all cast-iron pumps delivering a flow of up to 2500 gpm each.
- Water is continuously supplied for use either unfiltered, sand-filtered, or sub-micron filtered, and can be heated or cooled to within 0.3 deg C of the desired temperature.
- 14-hour light/ 10-hour dark photoperiod during animal acclimation and testing periods.
- Test containers used for solid phase bioassays are all-glass aquaria of 30 liters capacity with 1000 cm2 bottom area.

TEST ORGANISMS
- Macoma nasuta were collected from Tomales Bay. Clams were held in control sediment at ambient seawater temperature (13-15 deg C).
- At least 5 days prior to testing, the required number of animals were withdrawn from the holding tanks, placed in experimental aquaria with control sediment, and the temperature adjusted to 15 deg C.
- During holding, acclimation, and testing, the clams fed on phytoplankton and detritus present in Davenport seawater system; no additional food was provided.

TEST MATERIAL SAMPLING AND PREPARATION
- The drilling mud to be bioassayed was prepared and packed according to Region 2 procedures. Samples were stored at 2-4 deg C until preparation began.
- After preliminary pH testing and inspection, 22.7 liters of composited sample were transferred to a clean 190 liter polyethylene barrel and 90.8 liters of Davenport seawater were added.
- The pH of the resulting mud-seawater slurry was checked and found to be within 0.1 pH unit of ambient seawater.
- The mud-seawater slurry was mixed by vigorous aeration for 30 minutes.
- Following a one hour settling period the resulting elutriate (which required no centrifugation) was siphoned into clean buckets.
- The remaining sediment was reserved for use as the Solid Phase bioassay test material.

BIOASSAY TEST PROCEDURES
- Five replicates of sample and control treatments.
- A 3 cm layer of control mud was added to each tank, the tanks filled with water, and 20 Macoma nasuta added to each tank.
- After 48 hours of acclimation to the laboratory test environment, 1.5 cm of drilling mud was added to each sample treatment tank and an additional 1.5 cm layer of control mud was added to each control tank.
- A one hour settling period was allowed after sample addition, after which the flow-through seawater system was turned on.
- Solid phase bioassays continued for 10 days. At least twice each day, laboratory environmental control systems were checked for continuity.
- Daily measurements were made of system salinity and temperature and of the dissolved oxygen level in each aquarium.
- After the 10 day bioassay period, the contents of each tank were washed through a 3 mm plastic screen with seawater and the animals were retreived and counted.
- Test data were the number of survivors.

DATA ANALYSIS

- Solid Phase bioassays were analyzed by either Analysis of Variance or its non-parametric analogue for 2-sample comparison, the Mann-Whitney test.
- Variance homogeneity was the criteria which determined the appropriate analytical series (parametric or non-parametric).
- In all statistical tests, significance was based upon an alpha-level of 0.05.

Test substance : Drilling mud from Drilling Specialties Company containing Asphalt, sulfonated, sodium salt, CAS Number 68201-32-1 (XXXXXXXXXXX). Trade Name: Soltex.

Reliability : (1) valid without restriction
30.01.2004 (9)

5.1.1 ACUTE ORAL TOXICITY

Type	:	LD50
Species	:	rat
Strain	:	Sprague-Dawley
Sex	:	male/female
Number of animals	:	20
Vehicle	:	water
Value	:	> 5000 mg/kg bw
Method	:	EPA OPP 81-1
Year	:	1985
GLP	:	yes
Test substance	:	other TS

Result : LC50 was estimated to be greater than 5000 mg/kg bw in both male and female rats.

No animals were found dead during either the Dose Range or Single Dose Studies.

Clinical signs noted during the Dose Range Study were limited to instances of soft feces and/or a rough coat in all groups at one or more intervals.

Clinical signs noted in the Single Dose Study consisted of soft feces in three males and all females at one hour post dose, in all animals at two and four hours, and a rough coat in all animals on Day 1. All animals appeared normal from Day 2 through termination. All animals gained weight from initiation to termination.

Gross pathology findings noted in animals on the Dose Range Study were limited to pale adrenals in Groups 1-3 (1000 mg/kg, 2000 mg/kg, and 3000 mg/kg) and Group 5 (5000 mg/kg) males and in Group 2 (2000 mg/kg) and Groups 4-5 (4000 mg/kg and 5000 mg/kg) females and dark adrenals were noted in the Group 4 (4000 mg/kg) male. No observable gross pathology was noted in any of the Single Dose Study animals upon necropsy.

Source : Phillips Petroleum Company Acute Oral Toxicity Study in Rats - Product #2 - Final Report. Study performed by Hazleton Laboratories America Inc., Vienna Virginia for Drilling Specialties Company Bartlesville, Oklahoma.

Test condition : Test Animals:
- Young adult male and female albino rats (weighing between 200-300 grams) of the Sprague-Dawley strain.
- Maintained individually in elevated wire-mesh cages in temperature-controlled and humidity monitored quarters.
- Acclimation period of approximately one week.
- 12-hour light/dark cycle.

Methods:
- For Dose Range Study, one rat/sex was dosed at levels of 1000, 2000, 3000, 4000 and 5000 mg/kg bw (initial body weights of males ranged from 205.7 to 221.8 g, and the initial body weights of females ranged from 203.0 to 236.1 g).
- Five rats/sex were assigned to the Single Dose Study and were dosed at a level of 5000 mg/kg bw (initial body weights of the males ranged from 252.4 to 299.0 g, and the initial body weights of the females ranged from 227.6 to 264.7 g).
- The dosage factor was 20 ml/kg.

Preparation and Administration of Test Material:
- Distilled water was added to the test sample to bring it up to the desired volume.
- All mixtures were stirred during dosing.

Observations:
- Dose Range Study: each animal was observed for signs of toxic and pharmacologic effects at 1, 2, 4, 24, and 48 hours after test material administration.
- Single Dose Study: each animal was observed for signs of toxic and pharmacologic effects at 1, 2, and 4 hours after test material administration and once daily thereafter to 14 days.
- Mortality/moribundity was recorded twice daily.
- Individual body weights were recorded immediately prior to treatment and at termination in both studies and at Day 7 in the Single Dose Study.
- At the end of the study an acute oral LD50 was estimated for each sex.

Pathology: At termination of the Dose Range and Single Dose Studies, all rats were sacrificed by carbon dioxide asphyxiation and necropsied. Observations were recorded.

Test substance : Asphalt, sulfonated, sodium salt, CAS Number 68201-32-1. XXXXXXXXX purity. Trade Name: Soltex.

Reliability : (1) valid without restriction
Flag : Critical study for SIDS endpoint
30.01.2004 (4)

Type : LD50
Species : rat
Strain : Sprague-Dawley
Sex : male/female
Number of animals : 20
Vehicle : water
Value : > 5000 mg/kg bw
Method : EPA OPP 81-1
Year : 1985
GLP : yes
Test substance : other TS

Result : LD50 was estimated to be greater than 5000 mg/kg bw in both male and female rats.

No animals were found dead during either the Dose Range or Single Dose Studies.

Clinical signs noted during the Dose Range Study were limited to instances of soft feces and/or a rough coat in all groups. All rats appeared normal at termination.

Clinical signs were noted among all animals in the Single Dose Study and consisted of soft feces, a rough coat and/or red stains on the nose and/or eyes at one or more intervals during the study. All animals appeared normal from Day 3 through termination. All animals gained weight from initiation to termination.

Gross pathology findings noted in animals on the Dose Range Study were limited to bright red lungs in the group 4 (4000 mg/kg) female and pale adrenals in the Group 5 (5000 mg/kg) female. No observable gross pathology was noted in any of the Single Dose Study animals upon necropsy.

Source : Phillips Petroleum Company Acute Oral Toxicity Study in Rats - Product #5 - Final Report. Study performed by Hazleton Laboratories America Inc., Vienna Virginia for Drilling specialties Company Bartlesville, Oklahoma.

Test condition : Test Animals:
- Young adult male and female albino rats (weighing between 200-300 grams) of the Sprague-Dawley strain.
- Maintained individually in elevated wire-mesh cages in temperature-controlled and humidity monitored quarters.
- Acclimation period of approximately one week.
- 12-hour light/dark cycle.

Methods:
- For Dose Range Study, one rat/sex was dosed at levels of 1000, 2000, 3000, 4000 and 5000 mg/kg bw (initial body weights of males ranged from 209.0 to 233.8 g, and the initial body weights of females ranged from 222.4 to 232.1 g).
- Five rats/sex were assigned to the Single Dose Study and were dosed at a level of 5000 mg/kg bw (initial body weights of the males ranged from 290.6 to 300.8 g, and the initial body weights of the females ranged from 237.1 to 258.8 g).
- The dosage factor was 20 ml/kg.

Preparation and Administration of Test Material:
- Distilled water was added to the test sample to bring it up to the desired volume.
- All mixtures were stirred during dosing.

Observations:
- Dose Range Study: each animal was observed for signs of toxic and pharmacologic effects at 1, 2, 4, 24, and 48 hours after test material administration.
- Single Dose Study: each animal was observed for signs of toxic and pharmacologic effects at 1, 2, and 4 hours after test material administration and once daily thereafter to 14 days.
- Mortality/moribundity was recorded twice daily.
- Individual body weights were recorded immediately prior to treatment and at termination in both studies and at Day 7 in the Single Dose Study.
- At the end of the study an acute oral LD50 was estimated for each sex.

Pathology: At termination of the Dose Range and Single Dose Studies, all rats were sacrificed by carbon dioxide asphyxiation and necropsied. Observations were recorded.

Test substance : Asphalt, sulfonated, sodium salt, CAS Number 68201-32-1. XXXXXXXX purity. Trade Name: Soltex 31.

Reliability : (1) valid without restriction
Flag : Critical study for SIDS endpoint
15.01.2004

(5)

5.1.2 ACUTE INHALATION TOXICITY

5.1.3 ACUTE DERMAL TOXICITY

5.1.4 ACUTE TOXICITY, OTHER ROUTES

5.4 REPEATED DOSE TOXICITY

5.5 GENETIC TOXICITY 'IN VITRO'

5.6 GENETIC TOXICITY 'IN VITRO'

5.8 TOXICITY TO REPRODUCTION

5.9 DEVELOPMENTAL TOXICITY/TERATOGENICITY

(1) BCO Analytical Services B.V, 1997. Determination of the Partition Coefficient (n-octanol/water), HPLC method, of Soltex Shale Inhibitor, BCO project number 3096110462, Breda, The Netherlands.

(2) Chemex Environmental International Limited. 2002. The Toxicity to Turbot (Scophthalmus maximus) of Soltex Additive - Report for Drilling Specialties Company. Chemex reference ENV6103/050221. Cambridge, England.

(3) Chemex Environmental International Limited. 2003. The Bioaccumulation Potential of Sulphonated Asphalt Additive - Report for Drilling Specialties Company. Chemex reference ENV6222/100202. Cambridge, England.

(4) Hazleton Laboratories America, Inc. 1985. Acute Oral Toxicity Study in Rats - Product #2, Final Report. Project No. 2375-101. Vienna, Virginia.

(5) Hazleton Laboratories America, Inc. 1985. Acute Oral Toxicity Study in Rats - Product #5, Final Report. Project No. 2375-104. Vienna, Virginia.

(6) ISO/DIS 10253: Water quality. Marine algal growth inhibition test with Skeletonema costatum and Phaeodactylum tricornutum. Document no.: ISO/TC 147/SC5/WG5/N120. Nederlands Normalisatie-instituut, Delft (March 1991).

(7) Kooijman, S.A.L.M., A.O. Hanstveit, and H. Oldersma. 1983. Parametric Analysis of Population Growth in Bioassays. Water Research 17, pp. 527-538.

(8) Laboratory Technology, Inc. 1994. 96 Hour Range Finder Acute Toxicity Test of Drilling Fluid Suspended Particulate Phase - Based on Permit #: GMG290000. Lab Technology Control# DS019. Kenner, Louisiana.

(9) Marine Bioassy Laboratories. 1982. Drilling Mud Bioassay - Soltex - Acanthomysis sculpta and Macoma masuta - Prepared for IMCO Services (Houston Texas) and Drilling Specialties Company (Houston, Texas). Watsonville, California.

(10) OSPAR / PARCOM. 1995. Protocols on Methods for the Testing of Chemicals Used in the Offshore Industry.

(11) TNO Environmental and Energy Research. 1991. Effect of a 1% Soltex Solution (262-100-3) on the Growth of the Marine Alga Skeletonema costatum (ISO/DIS 10253), TNO Study no. IMW-91-0072-01. Delft, The Netherlands.

(12) TNO Environmental and Energy Research. 1991. The Biodegradability of the Product 3.5% Bentonite Slurry with 1% Soltex (262:100-2) in Seawater According to a Proposed EC Test Guideline (Closed Bottle Test), TNO Study no. IMW-91-0073-01. Delft, The Netherlands.

(13) TNO Environmental and Energy Research. 1991. The Biodegradability of the Product Soltex Shale Inhibitor in Seawater According to a Proposed EC Test Guideline (Closed Bottle Test), TNO Study no. IMW-91-0021-01. Delft, The Netherlands.

(14) ToxCalc Version 5.0 "Comprehensive Toxicity Data Analysis and Database Software," copyright 1994-1996.

(15) U.S. Environmental Protection Agency, 1993. Industrial Technology Division, March 4, 1993. Appendix 2 to Subpart A of Part 435 - Drilling Fluids Toxicity Test Regulation for offshore subcategory of the oil and gas extraction point source category, Federal Register Vol. 58, No. 41, 12507-12512 / Thursday, March 4, 1993 (40 CFR Part 435).

(16) United States Environmental Protection Agency, Office of Pollution Prevention and Toxics and Syracuse Research Corporation, 2000. EPI Suite v 3.10 (April, 2001).